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BIO-CHEMICAL DECOMPOSITION OF CELLULOSIC MATERIALS, WITH SPECIAL REFERENCE TO THE ACTION OF FUNGI[1]

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(With Plates I–IV, 7 Graphs and 1 Text-figure.)

CONTENTS.

I. INTRODUCTION.

THE problem of the decomposition of cellulosic materials has hitherto been attacked along two different lines. On the one hand there have been attempts to correlate the rate of decomposition with the chemical constituents of the materials. On the other hand, the discovery that micro-organisms are agents of such decomposition has led many investigators to direct their attention to the study of the behaviour of these organisms.

[1] Part of a Thesis Presented for the Degree of Doctor of Philosophy of London University.

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Wollny (44) found that amounts of nitrogen contained in organic materials controlled the rate of decomposition. Rahn (27) showed that the application of nitrate or ammoniacal nitrogen accelerated the decomposition of straw. This point was further developed by Hutchinson and Richards (16) when they first revealed the quantitative combination of nitrogen and carbohydrates. According to these workers, the chief necessity for the most rapid breakdown of straw is a supply of assimilable nitrogen compounds in suitable concentration under aerobic and neutral or slightly alkaline conditions. They further found that nitrogen thus applied in a soluble condition is temporarily immobilised, and that the amount of nitrogen that straw is thus capable of locking up is equal to that necessary for pronounced rotting. The amount of soluble nitrogen temporarily immobilised by 100 parts of dry straw or other cellulosic material is termed the "nitrogen factor." Richards and Amoore (29) found that even in spite of the addition of assimilable nitrogen, certain materials such as sawdust, rice-husk, old bracken or coconut-shell cannot be decomposed, while some others such as banana stem or hop-bines take a long time for decomposition.

Dvorak (10) stated that materials rich in oxygen and poor in carbon decomposed more rapidly than those rich in carbon and poor in oxygen.

Starkey (35) while acknowledging that the rapidity of decomposition of some crude organic materials, such as rye and alfalfa, may be associated to some extent with their nitrogen content, does not consider nitrogen to be the limiting factor. Dextrose was decomposed most rapidly of all the materials studied, some rich in nitrogen; and cellulose decomposed the most slowly. Thus, apparently, no broad generalisations have been found to apply to the relative ease of decomposition on the basis of carbon, nitrogen and oxygen contents.

A detailed study on various constituents of straw was carried out by Hebert (13) who found that they decomposed in the following order: chlorophyllous matter, gums, tannins, glucose, dextrin, higher carbohydrates (cellulose and straw-gum) and finally vasculose. Van Suchtelen (39) states that in general the less abundant hexoses and pentosans decompose first followed by the polysaccharides, celluloses, pectins, starches and albumins. A strongly resistant carbonaceous residue is left, which however is decomposed very slowly. Dvorak (10) finds that fresh plant materials are the most available largely because the lower carbohydrates are more abundant; in the older plants, ligno-celluloses predominate. Richards and Hutchinson (28) in their patent, claim that all carbonaceous materials containing an adequate total quantity of carbo-

hydrates (30 per cent. and upwards) such as starch and pentosans and preferably not too high a proportion of ligno-cellulose are fermentable in the presence of assimilable nitrogen.

The study of micro-organisms active in the decomposition of organic materials dates from the time of Mitscherlich (23) who is generally considered to be the first to attribute the decomposition of organic materials to bacteria. Since that time bacteria have been given a great deal of prominence, and till the beginning of this century, they were considered the sole agents responsible for the disappearance of organic matter in the soil.

A great deal of work has recently been done on the activities of fungi in relation to soil fertility, and though their activities appear, perhaps, to be not so varied as those of the bacteria, fungi are important contributors to the ammonification, nitrogen transformation, cellulose decomposition and humification processes. A detailed survey of the literature pertaining to these problems has been given by Waksman (40).

The problem of the decomposition of cellulosic materials is thus bound up not only with the investigation of the chemical constituents of the organic materials, but also with the behaviour of the micro-organisms towards these constituents. While the resistant nature of the compounds of nitrogen present in these materials has been definitely proved by many workers, information on the availability of the carbonaceous compounds during this process is incomplete. The present investigation is therefore directed to the study of the decomposition of carbonaceous compounds in presence of assimilable nitrogen, and also to the study of different micro-organisms most active in such decompositions as regards their behaviour towards these constituents.

II. Outline of the scheme of work.

The experimental work naturally falls into three parts:

A. *A quantitative study of different carbonaceous compounds during decomposition.*

The main carbon constituents of plants may be classified as (1) celluloses, (2) hemi-celluloses[1], (3) starches, (4) sugars, (5) pentosans, (6) lignins.

Two materials of different susceptibility to decomposition were selected for detailed study. One, rice-straw, is decomposed rapidly,

[1] No attempt is made to determine hemi-celluloses, as, though not of constant composition they mostly consist of pentosans, and these are separately estimated.

while the other, poplar wood, is attacked with difficulty by micro-organisms and is a good example of a resistant material.

In the case of rice-straw, the material was allowed to rot and periodical short time analyses were carried out for the above mentioned constituents as well as for ammoniacal and protein nitrogen. In the case of poplar wood different carbon compounds, for example sugars, starches and pentoses, were added and their effect on decomposition was observed. Various other materials were examined for their different carbon constituents, especially pentosans, celluloses and lignin, in order to discover a possible correlation between these and the "decomposibility" of the materials.

Other experiments were carried out under the same conditions with pure constituents. For this purpose pure cellulose, in the form of filter paper, was moistened with purified preparations of hexoses and pentoses. The experiment was intended to indicate which of these constituents might assist the decomposition of raw materials.

B. *A study of the relative importance of bacteria and fungi in decomposition.*

Bacteria and fungi were isolated from a decomposed manure heap and were separately inoculated into the rice-straw. The results of their activities were quantitatively determined and the products of decomposition were submitted to nitrification tests.

C. *A study of the life history and physiological behaviour of certain fungi which were found (vide section B) to play the more important part in the process.*

Three fungi very active in these processes were isolated and their morphology and physiology were studied especially in relation to plant constituents.

III. Methods of analysis.

The following methods of analysis were adopted throughout the experimental work.

Furfuroids. The standard method, Krober and Tollens (1), for the determination of pentoses and pentosans was employed. The furfurol obtained by distillation was estimated as phloroglucide. All figures are expressed as pentosans.

Dextrose as well as *Invert Sugars* were estimated in the alcoholic extracts by the iodimetric method (1).

Starch was at first determined by the hydrolysis method (1). Two gm. of the material, well washed to free it from reducing sugars, were heated on the water bath from two to five hours with 200 c.c. of water and 20 c.c. of hydrochloric acid (sp. gr. 1·125). A portion of the filtrate was distilled with hydrochloric acid for pentosans, while in the other portion, the copper reducing power was determined by the iodimetric method. The copper reducing power of starch was obtained by subtracting the equivalent of pentosans from the total, but compared with the Taka-diastase method (9) the figure thus obtained was higher—perhaps as a result of the hydrolysis of some other constituent. In the case of fermented materials both these methods gave quite untrustworthy results.

Water-soluble and alkali-soluble portions (11). Two gm. of the material were heated on the water bath for one hour with 100 c.c. of distilled water, filtered, washed with hot water, dried and weighed. The loss in weight gives the figure for water soluble substances. The residue was treated on the water bath for half an hour with 1 per cent. alkali (treatment as in the chlorination method for cellulose estimation), filtered, washed with dilute acetic acid and then with hot water till free from acid, dried and weighed. The difference between this weight and that of the water-soluble portion represents alkali-soluble substances.

Cellulose was estimated by the chlorination method of Cross and Bevan (8). Two gm. of the roughly ground material were heated on the water bath for 20 minutes with 100 c.c. of 1 per cent. NaOH solution, the volume throughout being kept constant by the addition of water. Owing to the difficulty in filtration, especially with decomposed materials, the Gooch crucible in Sieber and Walter's (33) apparatus was replaced by a small Buchner funnel. Three chlorinations of 15, 7 and 5 minutes were found sufficient for the purification of cellulose. After each chlorination a little sulphurous acid was added to stop any further action of chlorine, the excess being removed by filtration and washing. The contents were then washed into a beaker with a jet of hot water, made up to known volume and heated on the water bath for 30 minutes with 3 per cent. sodium sulphite solution. After the third chlorination and treatment with sodium sulphite, it was filtered through a Gooch crucible and thoroughly washed. The contents were then flooded with 2 per cent. permanganate solution for five minutes, filtered, washed, treated with sulphurous acid and finally washed until free from acid. The residue from undecomposed materials was white. In the fermented materials, the first chlorination turned it darker, the colour being reduced with further chlorinations. But in the end it retained a greyish

colour which was constant in spite of either additional chlorinations or treatment with permanganate and sulphurous acid. Its freedom from lignin could be verified by treatment with 42 per cent. hydrochloric acid.

It was found inadvisable to have the material finely ground for chlorinations. Besides causing difficulties in filtration, the particles, owing to their fine nature, came in very close contact with each other, thus making the free passage of chlorine very difficult. In such cases even three chlorinations were found insufficient for the removal of impurities.

All the figures are calculated on the cellulose free from ash.

Lignin. Attempts were made at first to get the figure for lignin by difference. After the alkali treatment in the chlorination method, the residue was dried in the steam oven and weighed. The difference between this weight and that obtained after chlorination was considered to represent lignin. But as this was found to give very low figures it was replaced by Willstatter and Zechmeister's method (41). The figures are calculated on lignin free from ash.

Total Nitrogen (1) was determined by the Kjeldahl method, while for ammoniacal nitrogen (1), the material was distilled with magnesium oxide.

IV. Experimental.

A. *A quantitative study of different carbonaceous compounds during decomposition.*

Series I. Decomposition of rice-straw.

A preliminary analysis of rice-straw gave the following results:

Dry matter	100 gm.	Invert sugar	0·6 gm.
Organic matter	82·67 ,,	Pentosans	24·6 ,,
Cellulose	45·46 ,,	Lignin	10·06 ,,
Starch	0·7 ,,	Total nitrogen	0·41 ,,
Dextrose	0·65 ,,		

A number of preliminary experiments in decomposition were carried out to ascertain the best temperature, the best interval between two analyses and the most important constituents requiring special attention.

A comparative study at two temperatures of 35° and 50° C. showed the former to be suitable for investigation, the decomposition being slow enough to enable the disappearance of various constituents to be traced. Further, it was also found that an interval of about four days between analyses was essential to give an idea of the relative importance of various compounds.

During the course of these experiments, it was found that the determination of sugars did not give a fair index of their disappearance during the course of decomposition due to the formation of these sugars as intermediate compounds. To measure their importance, the alternative method of elimination had therefore to be employed. It was thus found that the removal of sugars by cold water lixiviation, which extracted 93 per cent. of the total quantity, had no effect on the rate of decomposition.

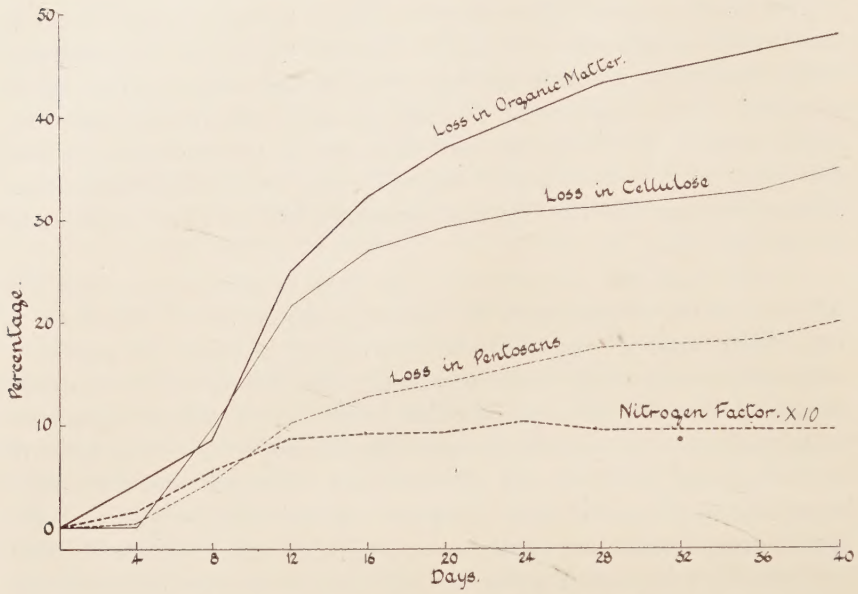
As regards starch, its presence in the straw in minute quantities, the difficulty of its estimation in the rotting materials due to the lack of any reliable method, as well as the impossibility of using the method of elimination which in this case necessitates autoclaving with consequent structural changes, force us at present to give up the idea of tracing its decomposition. The resistant nature of lignin shown by various workers as Mahood and Cable (20) and Johnsen and Hovey (17) proves its non-importance as microbial food. Thus the only constituents which require careful consideration are pentosans and cellulose and in this series their decomposition is studied under the best conditions of temperature and period of analyses. The procedure was as follows.

Twenty gm. of chaffed rice-straw, obtained from the Punjab, were accurately weighed, slightly moistened with water by a sprayer and allowed to remain in this condition for about 10 minutes. Ammonium carbonate solution, equivalent to about 1 gm. of nitrogen (16) to 100 gm. of straw was added drop by drop from a burette and thoroughly mixed with the straw. After the addition of inoculum from old decomposed manure the straw was bottled and enough water was sprayed to cause thorough wetting. The bottles were incubated at 35° C. and analyses were carried out every four days. The portions for ammoniacal nitrogen, total nitrogen and dry weight were taken immediately. The rest of the material was thoroughly dried in the steam oven and kept for other determinations.

The fungi were seen on or about the 7th day. They continued their vigorous growth till the 20th day when they began to sporulate. By the 24th day sporulation was complete, and by the 28th day, no trace of fungus hyphae could be seen by the naked eye. Microscopic examination on the 36th day showed no hyphae, though spores were observed in plenty. No water-logging was observed except in one case.

The results are given in Table I and also represented graphically.

Discussion. It is quite evident from the results, that during the first fortnight there is a rapid loss of dry matter as well as assimilation of



Graph 1. Decomposition of Rice-Straw at 35° C.

Table I.

Decomposition of rice-straw at 35° C.

Figures calculated on 100 gm. of original dry matter.

	At start	4 days	8 days	12 days	16 days	20 days	24 days	28 days	32 days*	36 days	40 days
Dry matter	100	95·57	91·2	75·4	69·8	63·9	60·8	58·6	52·9	56·8	55·0
Organic matter	82·67	78·37	74·1	57·8	50·65	45·8	43·03	40·05	33·9	37·0	35·5
Total nitrogen	{0·41 / 1·13}	1·3	1·38	1·37	1·35	1·35	1·45	1·36	1·31	1·36	1·33
Ammonia nitrogen	1·13†	0·78	0·44	0·086	0·037	0·024	0·014	0·019	0·059	0·021	0·023
Nitrogen factor‡	—	0·12	0·53	0·88	0·90	0·92	1·02	0·93	0·84	0·91	0·90
Pentosans	24·78	24·65	20·6	14·33	12·5	10·65	9·08	7·42	Not determined	6·8	5·32
Fraction soluble in water	14·1	14·95	12·8	16·4	15·1	13·9	13·2	13·25	,,	12·3	11·7
Fraction soluble in alkali	32·2	26·85	34·9	27·3	26·9	23·6	23·0	20·55	,,	20·5	20·3
Residue	53·7	53·7	43·5	31·7	27·8	26·4	24·6	24·8	,,	24·0	23·0
Cellulose	45·46	45·88	35·8	24·05	18·7	16·75	15·17	14·8	,,	13·1	11·1
Fungus growth	—	Nil	Just appearing	Vigorous	Vigorous	Vigorous	Sporulating	Spores	Not visible	Not visible	Not visible

* Water-logged. H_2S smell.

† Added as $(NH_4)_2CO_3$.

‡ This is the amount of available nitrogen temporarily immobilised by 100 gm. of straw (16).

ammonia. The visual inspection of bottles showed the presence of fungus hyphae on the 6th or 7th day and this is immediately followed by the rapid loss of dry matter as the figure for the 12th day indicates. Table I and Graph I clearly indicate that so long as the fungi were vigorously growing, disappearance of dry matter and assimilation of ammonia were at their highest. More than 60 per cent. of the dry matter was lost and nearly 90 per cent. of the ammonia was assimilated during that period.

Further, the loss of pentosans shows a striking correlation with the fungus growth. (The physiological studies of fungi show that cellulose is a poor nutrient food for them.) During the first four days, there is no loss of pentosans at all, but between the 4th and 8th day, when the fungus growth must have started, the loss of dry matter is represented almost wholly by that of pentosans (see Graph 1). It seems, from the results after the 12th day, that the fungi have a tendency to store these pentosans. *Coprinus* sp. separated from the decomposing straw gave 7·82 per cent. pentosans on dry weight. During the period of vigorous destruction of straw this fungus alone is found to form an appreciable part of the whole material. In one case 151 gm. of the wet material gave 17 gm. of this fungus or nearly 11 per cent. Thus pentosans seem to be a very suitable food for fungus growth.

It must not be argued from this that pentosans are the chief food materials for fungi. Later work shows that they grow equally, and in some cases even better, on some sugars and starches, but as a rule, waste plant materials contain very little of these; indeed rice-straw is very deficient in both. It can therefore be safely concluded that fungi are playing an important part in this process.

As regards losses of various constituents, more than 80 per cent. of the pentosans disappeared during the 40 days of the experiment. The loss of pentosans, especially during the early stages of decomposition, is striking, and suggests, as discussed above, the importance of these compounds for the activities of micro-organisms. Cellulose has also suffered a great loss. Apparently the loss in cellulose is even greater than in pentosans. But the study of Table II shows that real loss is less as the rapid loss of cellulose is followed by an increase in the alkali and water-soluble portions. It is, therefore, quite likely that cellulose passes through these stages of decomposition.

Lisse (19) in his study of rotting wood found the increase to occur in the alkali-soluble portion which according to him indicates the formation of hemi-cellulose. Bray and Staidal (3), in their study of the chemical

Table II.

Showing the stages through which cellulose passes during decomposition.

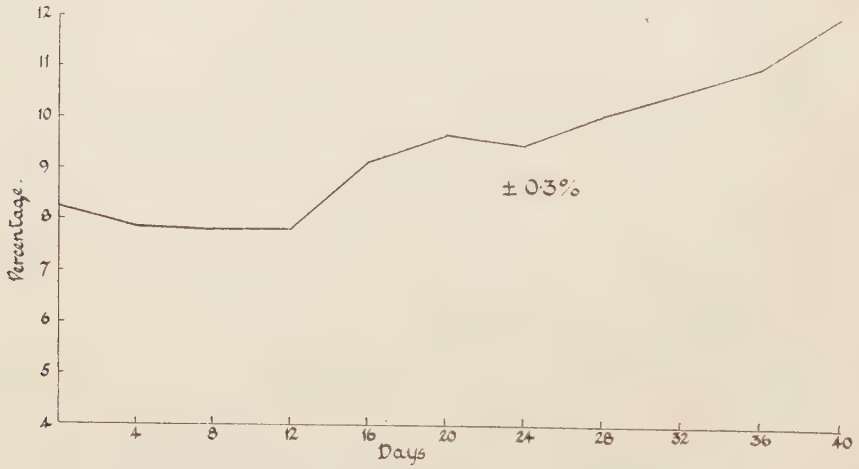
Calculated on 100 gm. of original dry matter.

Days	Loss in cellulose	Loss or gain in water and alkali-soluble portions	Total loss or gain	Loss in organic matter
4	0	−4·5	− 4·5	− 4·3
8	−10	+6·0	− 4·0	− 4·27
12	−11·9	−4·0	−15·9	−16·3

N.B. Since at later stages of decomposition the cellulose itself suffered loss during analysis, the figures for these values after the 12th day are not given.

changes involved during infection and decay of wood and wood-pulp, found an increase in cold and hot water-soluble portions as well as in alkali-soluble associated with a decrease in the amount of cellulose. Various authors who have devoted their attention to the decay of wood-pulp find that the highly complex celluloses, *e.g.* α-cellulose, pass into others of increasing simplicity as β- or γ-cellulose or hemi-celluloses, till at last all pass into CO_2, water and perhaps hydrogen and methane.

Further, the gradual increase observable after the 16th day in the difference between the residue after alkali treatment and the cellulose



Graph 2. Percentage difference between residue after alkali treatment and cellulose after chlorination, showing that at later stages of decomposition cellulose is attacked by chlorine.

figure obtained after chlorination proves that cellulose becomes less resistant to this chlorination treatment during decomposition. As this difference mostly represents lignin, the other constituents being almost

removed by alkali treatment, it should never show such an increase. On the other hand, accompanying the breakdown process, there should be a decrease as the humus formed from lignin (12) is easily removable by treatment with alkali. As indicated in the description of the method of chlorination, the material gets darker with the first chlorination, and though the colour is reduced with successive chlorinations, the residue is never white as in the case of original straw. It seems, therefore, that the cellulose itself becomes weaker and more easily susceptible to the attack of chlorine. Consequently the figure obtained represents less cellulose than is actually present in the material and it is very difficult to get an idea of the real loss of this constituent. None the less, it definitely shows that the greater portion of cellulose is not resistant at all and is even susceptible to the attack of micro-organisms during the first stages of decomposition.

Series II. Decomposition of poplar wood.

In this series attempts were made to decompose woody material. Poplar wood was chosen for experiments. These experiments confirmed the general experience that the woods are not easily attacked by micro-organisms even in the presence of available nitrogen.

A preliminary analysis of poplar wood for important constituents gave the following results:

Dry matter	100·0 gm.	Pentosans	20·63 gm.
Organic matter	98·32 ,,	Lignin	28·44 ,,
Cellulose	66·3 ,,	Total nitrogen	0·31 ,,

As is evident from the above table, there is a certain overlapping in the figures for the different constituents, *e.g.* in the case of pentosans and cellulose. The same may be expected in the case of rice-straw.

The experimental technique for the decomposition of this material was exactly the same as before. 20 gm. of the wood-shavings were used for each experiment and available nitrogen was added in the form of ammonium carbonate. As woods are rather deficient in ash, a definite quantity of mineral salt solution[1] was added. A control set was incubated at the same time. To both, 1 gm. of calcium carbonate was added.

The bottles with mineral salts showed the fungus growth on about the 6th day, while those without showed only a slight growth after a

[1] Composition of mineral salt solution:

$NaNO_3$	2·5 gm.	$CaCl_2$	0·1 gm.
K_2HPO_4	1·0 ,,	$FeCl_3$	0·02 ,,
$MgSO_4.7H_2O$	0·3 ,,	Distilled water	1000 c.c.
NaCl	0·1		

fortnight. In none of the bottles was the action as vigorous as with rice-straw. The experiment was stopped after two months and the contents were analysed as in the previous series.

Table III.

Decomposition of poplar wood at 35° C., for two months.

Figures calculated on 100 gm. of original dry matter.

	Wood gm.	Wood + mineral salts gm.
Loss of organic matter	19·77	37·14
Ammonia nitrogen	0·11	0·036
Total nitrogen	0·68	0·96
Nitrogen factor	0·26	0·62
Loss of pentosans	—	46·1
Loss of cellulose	—	63·2

Similar experiments, made with pine wood shavings, gave no evidence of decomposition even after two months: after this period the loss of dry matter was found to be about 3 per cent. No further analysis was therefore made.

Discussion. These results clearly show that lack of minerals is one of the essential factors inhibiting decomposition. The addition of minerals alone has almost doubled the loss of dry matter and more than doubled the nitrogen factor. This again lends support to the theory that fungi play an important part in the decomposition. The controls showed very poor growth of fungus flora and the loss of dry matter as well as the nitrogen factor are quite insignificant.

As regards loss in various constituents, that in cellulose should be accepted with qualification for the reasons already stated. But even after making allowances for these objections, cellulose has suffered a great loss, even greater than pentosans. The small loss in pentosans is rather surprising. According to the previous experiment with rice-straw, pentosans suffer the greatest loss during the early stages of the process; but with poplar wood, this does not appear to be the case, for even after two months' fermentation only 50 per cent. of the pentosans has disappeared. Two possible explanations may be advanced.

(1) *The difficulty experienced by organisms in getting access to the food materials.* It is a fact well established, that the cell-walls of wood and straw are lignified, the amount of lignification varying greatly in different groups of higher plants. For instance, woods are far more lignified than straws. The process of lignification, according to Sachs (31), consists in a series of progressive and intrinsic modifications of a cellulose or oxy-

cellulose tissue, the final products of metabolism (aromatic products, pentosans, etc.) being split off from the fundamental tissue substances and excreted. The distribution of these non-cellulosic constituents is at present a controversial point. Wislicenus (43) has tried to explain it by the adsorption theory, while there is another school of thought which, both by the study of the reactive groupings of the constituents of the ligno-cellulose (21) as well as the resistance offered by these non-cellulosic products to chemical treatments (32), considers these compounds to be chemically combined with cellulose. It suffices our purpose to know that the products of metabolism of the cell-wall remain intimately associated with the residues of the fundamental tissue. It is thus quite reasonable to conclude that micro-organisms have to attack this fundamental tissue to get their nourishment. Thaysen and Bunker (36), who have made a microscopic study of the destruction of cellulose fibres and fabrics by micro-organisms, come to the conclusion that destruction is from outside inwards. A microscopic study of sections of wood, inoculated with soil suspension, shows that organisms pierce the tissue at the point at which they happen to be present, and their appearance in the cell-wall itself supports the assumption that they do not obtain their food from a distance by enzymic activity.

Of the fundamental tissue, lignin is the most resistant, and therefore the rate of decomposition should be dependent upon the quantity of lignin present. The slow rate of decomposition of poplar wood and other woody materials may therefore be attributed to their high lignin content. Due to this resistant barrier, micro-organisms in woody tissues cannot easily obtain their food materials—pentosans. In the course of their activities, they attack the least resistant of the fundamental tissue components, viz. cellulose. This is probably the reason of the great loss of cellulose found during decomposition of poplar wood. But the slow activity of these organisms indicates that cellulose is not as good a food as some lower carbohydrates. The increased decomposition of plant tissues when in a state of fine division lends support to the theory.

To the above theory, however, there is one objection which is of a practical nature. In actual decomposition it appears that the rate of breakdown is not solely dependent upon the lignin content. This can be seen in the case of pine and poplar woods. The lignin content of poplar and pine respectively is 28·44 and 26·65 per cent. But while poplar comes under the category of slow decomposing materials, pine is hardly attacked at all. Thus there appears to be an additional inhibitive factor in pine wood. Whether this may be the lack of some necessary

food material is discussed below. The possibility that the resinous compounds of pine may prevent the action of organisms has not been overlooked, but the works of Soderbaum and Barthel(34) and also of Migula(22) suggest that this may not be of importance.

(2) *Nature of furfurol-yielding constituents.* In all previous experiments, the term "pentosans" is considered to be identical with furfurol-yielding compounds. But analytical conversion into furfurol, while specially characteristic of the pentoses and pentosans, is also a property of certain higher compounds such as glycuronic acid and probably oxycelluloses. It is claimed(8) that these types of cellulose, which are much more widely distributed in the plant world than the cotton type, yield furfurol as a product of hydrolysis by hydrochloric acid. For example, 8·9 gm. of pentosans were obtained from the cellulose of 100 gm. of rice-straw, *i.e.* 33 per cent. of the total pentosans in this case is represented by some portion of the cellulose.

Though this differentiation may not be important chemically, it is likely to have a great physiological significance. This can be best illustrated by certain fermentation tests on the furfuroids. Cross, Bevan and Smith(7) in their researches on the carbohydrates of cereal straws have obtained the following interesting results.

Barley straw from Rothamsted plots was digested with 2 per cent. sulphuric acid under pressure and yielded an extract containing 70–80 per cent. of the total furfuroids in the straw. This was neutralised and fermented with yeast.

Furfuraldehyde	(before fermentation)	... ...	38·0
,,	(after fermentation)	... ...	4·7

Similar fermentation of the furfuroids obtained by hydrolysis of the straw cellulose with the same strength of acid at three and a half atmospheres pressure for 15 minutes gave the following results:

Furfuraldehyde	(before fermentation)	... ...	39·7
,,	(after fermentation)	... ...	26·1

Thus the hydrolysis product obtained in the two cases, though chemically similar, is not fermentable at the same rate. We have no definite idea as to the nature of the original material which has suffered this hydrolysis. So far, no method isolates it, in its original state, from cellulose. It is not, therefore, possible to get any direct evidence of its resistance to microbial attack. But we can come indirectly to certain conclusions by studying how these furfurol-yielding bodies behave during decomposition.

The figures in Table IV were obtained as a result of the experiment in Series I and II. In the case of rice-straw in Series I estimation of pentosans in cellulose was done only on the 8th and 12th day as those days showed the greatest loss in cellulose.

Table IV.

Loss of pentosans and cellulose at various stages of decomposition.

	Days	Loss of cellulose %	Loss of pentosans in cellulose %
Rice-straw (Series I)	8	21·3	9·0
,, ,, ...	12	45·0	40·0
,, ,, ...	40	75·6	73·0
Poplar wood (Series II)	60	63·2	52·4
Poplar wood rice-straw (Series III)	60	73·2	72·8

Table IV indicates that cellulose is even less resistant than the furfurol-yielding bodies. Bray and Andrews (4) in their study of chemical changes of ground wood during decay also found that the loss in cellulose was greater than the loss in total pentosans. After three years' decomposition the figure for cellulose dropped down from 60 to 6·05 per cent. while that for pentosans was reduced from 12 to 2·56 per cent.

The chemical behaviour of these furfurol-yielding bodies also shows their resistibility. Heuser and Haug (14) found that treatment with boiling 6 per cent. caustic soda did not reduce the xylan content of straw below 9·7 per cent. According to Winterstein (42) boiling with 5 per cent. sulphuric acid for three hours did not remove all the xylan. Although Heuser and Boedeker (15) have claimed to free the cellulose from pentosans by repeated extraction with hot 6 per cent. or cold 17 per cent. caustic soda, this drastic treatment led to the destruction of a considerable part of cellulose. Thus all the evidence, direct as well as indirect, points to the conclusion that these furfurol-yielding bodies are more resistant than the true pentosans and perhaps some celluloses. As shown before, micro-organisms thrive well on available pentosans, and increase in resistant furfurol-yielding bodies, therefore, would not be conducive to rapid decomposition. This is actually found in the case of poplar wood. Though the wood shows a large amount of total pentosans, about 50 per cent. of these belong to the category of resistant bodies. It would seem probable that this is the reason why even after two months such a large amount of pentosans is found in the decomposing material. In the rice-straw, on the other hand, less than 34 per cent. of the furfuroids are in this resistant form, so that more than

66 per cent. may be regarded as easily available. It would be, therefore, reasonable to conclude that rice-straw would be more rapidly destroyed than poplar wood and actual tests support this view.

Extending the above theory further, we can postulate that the food factor is the limiting factor in the decomposition of the material, or in other words susceptibility to destruction is proportional to the amount of available food in any material. But though this is true in many cases, it is not applicable to all cellulosic materials. Some of these, containing equal amounts of assimilable pentosans, show great divergence in their susceptibility to decomposition, *e.g.* rice-straw and maize-straw. Both contain equal amounts of pentosans, but while the former is easily destroyed the latter is decomposed less easily. Thus we see that the food factor, like the inhibitive factor, though important, is not the only condition controlling decompositions of this nature.

It is quite clear from the above discussion that these two factors—inhibitive and food—are not independent. Both must be acting simultaneously and the predominance of the one above the other would determine rapid breakdown or otherwise of the material. In other words, if pentosans are much in excess of lignin, the material will be easily

Table V.

Analysis of plant materials showing the relation of the ratio of pentosans and lignin to their "decomposibility."

Figures expressed on 100 gm. of dry matter.

	Total furfuroids expressed as pento-sans*	Cellulose by chlor-ination method	Furfuroids in cellu-lose as pentosans	Available furfuroids as pento-sans*	Lignin	Ratio of available pentosans to lignin	Decom-posability
Rice-straw	24·78	45·88	8·9	15·88	10·31	1·54	Rapid
Oat-straw	28·8	51·7	12·8	16·0	14·18	1·13	,,
Barley-straw	28·4	49·4	11·2	17·2	16·16	1·06	,,
Wheat-straw	31·28	52·1	15·15	15·13	14·57	1·04	,,
Rushes	27·93	51·3	12·7	15·23	17·71	0·86	Slow
Maize-straw	25·83	45·0	10·6	15·23	20·34	0·75	,,
White ash	22·33	53·4	9·26	12·07	28·38	0·43	Very slow
Poplar	20·63	66·3	10·95	9·68	28·44	0·34	,,
Pine	8·97	57·41	5·9	3·07	26·65	0·12	Nil

* Although "available" pentosans calculated as above are on the whole in the same order as total furfuroids, it is found that the actual order of "decomposability" runs more closely parallel with the "available" pentosans than the total furfuroids. A case in point is that of barley-straw. In this case while the ratio in the table puts it in the category of "rapid" which is also confirmed by actual tests, the ratio of total furfuroids to lignin would include it among the slowly decomposed materials.

decomposed, the rate of its decomposition decreasing as the ratio of pentosans to lignin decreases. If this be true, it should be easy to discover an index of the "decomposability" of a material by ascertaining the relative amounts of available pentosans (*i.e.* furfurol-yielding bodies obtained by deducting furfuroids of cellulose from total furfuroids) and lignin. In Table V some of the materials are tested to ascertain this ratio as well as their "decomposibility."

The results indicate that this test, while not giving exact figures, is quite satisfactory. It is possible to judge that any material having a ratio higher than 1·0 can be easily decomposed by micro-organisms; if the ratio falls between 1·0 and 0·5, the material is rather slow and so on. It is thus quite definite that both the factors—food and inhibitory—are inter-related, and that the presence of both, in certain definite ratios to each other, controls the rate of decomposition of plant materials.

Series III. Effect of carbohydrates on the decomposition of wood.

The previous experiments seem to prove that for a material to be easily decomposed the ratio of pentosan to lignin must be higher than 1·0. In this series attempts were, therefore, made to increase this ratio in poplar wood by the addition of various carbohydrates. The experimental technique was the same as before. Twenty gm. of the wood shavings were used for each bottle and each received 10 c.c. of mineral salt solution and 1 gm. of calcium carbonate. Straw gum was isolated by Tollens and Wheeler's(38) method. The arrangement of the series was as follows:

(1) Dextrose ... ... 1 gm. per bottle.
(2) Starch ... ... 1 ,,
(3) Xylose and arabinose in equal proportions 1 ,,
(4) Straw gum ... ... 1 ,,
(5) Rice-straw ... ... 5 ,,

Table VI.

Decomposition of poplar wood at 35° C. for two months.

Figures calculated on 100 gm. of original dry matter.

	Dextrose	Starch	Xylose arabinose	Wood gum	Rice-straw	Minerals only
Loss of organic matter	35·34	27·19	31·1	33·3	54·5	37·14
Ammonia nitrogen	0·08	0·09	0·08	0·096	0·007	0·036
Total nitrogen	0·83	0·73	0·79	1·12	1·004	0·96
Nitrogen factor	0·45	0·33	0·41	0·71	0·69	0·62
Loss of pentosans	—	—	—	—	72·6	46·1
Loss of cellulose	—	—	—	—	73·2	63·2

All the cultures showed the fungus growth, but there was no visible decomposition except in the case of the rice-straw, which also retained moisture more efficiently than the others. The experiment was stopped at the end of two months and the materials were analysed (Table VI).

Discussion. The results are rather disappointing. The addition of food materials outside the tissue lowers the actual decomposition of the tissues. The loss of organic matter in all except rice-straw is less than that with minerals only. It seems therefore that the micro-organisms which obtained both their nitrogenous and carbonaceous food outside the tissues did not attack that lying within till this easily available supply was finished.

This is shown by the great loss that took place with rice-straw. In this case it was essential for the organisms to attack the tissues, and though they might have first attacked the straw, they afterwards attacked straw and wood equally. In fact, the material after two months was found to be a mass in which straw and wood were closely intertwined together by fungus hyphae. Even after deducting the figure for the loss of organic matter in which straw may be taken as 55 per cent. within two months, the wood alone shows a loss of 53·25 per cent. in this experiment. Thus though carbohydrates form good microbial food, their addition to plant materials does not in any way help decomposition of the latter.

Series IV. Importance of hemi-cellulose in the decomposition.

We have left one important group of plant constituents called hemi-celluloses out of account because their composition is a matter of great controversy. It is so far known that these substances closely resemble the true celluloses, but are easily resolved into simpler carbohydrates by the hydrolytic action of enzymes and of dilute acids and alkalis. The carbohydrates thus obtained are xylose, arabinose, mannose and galactose. In our studies on decomposition we have given careful attention to the first two and as a result have come to the conclusion that they are important as microbial food. But our neglect of the other two would be a possible source of criticism against our theory, as they form in many plants an appreciable part of the hemi-celluloses. In fact, in the absence of any proof to the contrary it might be rightly claimed that hemi-celluloses and not pentosans alone may be the determining factor in the decomposition processes. This series is an attempt to test the validity of such criticism.

The difficulties in tracing the decomposition of hemi-celluloses are

obvious. Not only are these compounds complex materials of uncertain definition, but they differ in composition greatly in different plants. Thus the study of their decomposition in any one material would not lead us any further. It was therefore thought best to study the behaviour of their purified hydrolytic products. Thus xylose, arabinose, galactose and mannose were obtained in pure form and their effect on the decomposition of cellulose (filter-paper) was tested. The method of procedure was as in the last series. 15 gm. of chopped Whatman's filter-paper No. 1 were used for each bottle. These were moistened with 10 c.c. of mineral salt solution. Nitrogen was added in the form of ammonium carbonate. Carbohydrates were added in two doses to prevent high concentration. The plan of the experiment was as follows:

(1) Control (filter-paper)
(2) ,, Mannose 0·75 gm. per bottle
(3) ,, Galactose ,,
(4) ,, Xylose ,,
(5) ,, Arabinose ,,

All the bottles received 1 gm. of calcium carbonate. They were inoculated with a suspension from decomposing manure and incubated at 35° C. All except the control bottle showed fungus growth after about the 12th day. The control showed slight fungus growth after a month, but the decomposition in this was more rapid than in the other bottles. It continued to be vigorous all the time and at the end of the period (six months) only a small quantity of the material remained in the bottle. The decomposition in other materials was very slow. The series was stopped after six months and analysed. Except in the case of arabinose, all, including the control, had retained the original colour of the filter-paper. Arabinose showed browning of the residual material. The results are given in Table VII.

Table VII.

Decomposition of cellulose in presence of carbohydrates at 35° C., for six months.

Figures calculated on 100 gm. of original dry matter.

	Control	Mannose	Galactose	Xylose	Arabinose
Loss of organic matter	82·1	19·15	22·2	39·8	48·8
Ammonia nitrogen	—	0·012	0·043	0·011	0·012
Total nitrogen	0·67	0·29	0·31	0·54	0·58
Nitrogen factor	0·63	0·24	0·23	0·49	0·53

Discussion. (1) Mannose and galactose do not seem to be good microbial foods for the cellulose decomposing organisms under these conditions. The loss in organic matter and the nitrogen factor in both cases are very low. The low nitrogen factor, especially, indicates that the micro-organisms have not shown much activity in growth. Thus these compounds do not appear important in such decomposition under these conditions and their omission from our consideration would seem to be justified.

(2) This series supports the conclusion drawn from the previous one as regards the low rate of "decomposability" of the material when both carbon and nitrogen compounds are added. The micro-organisms do not attack the tissue in the presence of other more available food material.

B. *A study of the importance of different groups of micro-organisms concerned in the process.*

In this section, attempts are made to investigate the relative importance of bacteria and fungi in the decomposition of plant materials. For this purpose sterilised straw was inoculated with pure cultures and the products of the activities of the several groups of micro-organisms were quantitatively estimated.

Series V. Decomposition of rice-straw by soil flora.

This series was devoted to the study of the combined activities of the soil population in the decomposition of rice-straw. It has been described in detail in Series I.

Series VI. Decomposition of rice-straw by fungi.

In this series, fungi in pure culture were inoculated into sterile straw and the products of their activities were analysed at periodic intervals as in previous series.

Twenty gm. of rice-straw were autoclaved in bottles under 15 lbs. pressure for half an hour on four consecutive days. In spite of the danger of changes in the straw, such autoclaving was found essential for complete sterilisation, steaming being insufficient to kill all the bacteria. Ammonium carbonate solution, in slight excess to what was theoretically required (1 part of nitrogen to 100 parts of dry matter), was diluted in separate flasks with water, just sufficient to wet the straw thoroughly and sterilised at 15 lbs. pressure for half an hour. It was found by a preliminary experiment that very little ammonia is volatilised in this way. These flasks were inoculated with the cultures

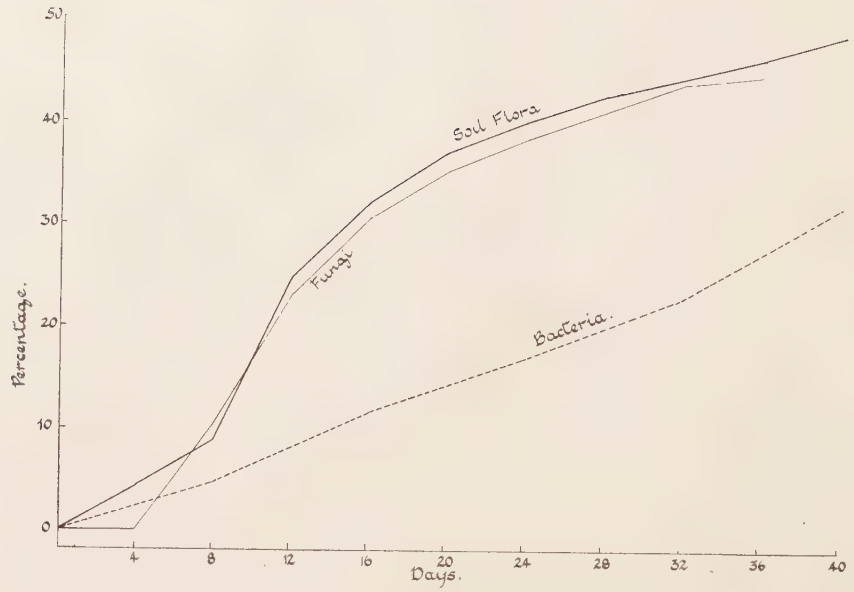
of three fungi (described in part C) grown on potato agar at 35° C.; they were then shaken for a few minutes and the contents were poured separately into each bottle. The inoculum consisted of spores in the case of *Aspergillus* sp. and *Acremoniella* sp. and mycelium in the case of *Coprinus* sp. The bottles were incubated at 35° C. and analyses were carried out every four days. At the time of each analysis care was taken to test the material for bacterial infection, both microscopically and by plating on Thornton's medium (37).

Fungi were visible on about the 6th day and continued their vigorous growth till about the 20th day. In all the bottles *Coprinus* predominated and the mycelium was so intimately intertwined with the material as to form one solid mass. In the case of the material decomposed by soil micro-flora, the fungus mycelium disappeared after about 32 days but in this case, even after the completion of its vigorous growth, the mycelium retained this state during the rest of the period. The whole experimental period in this case as in previous experiments was 40 days. Two cases only showed bacterial infection and these were therefore omitted from consideration.

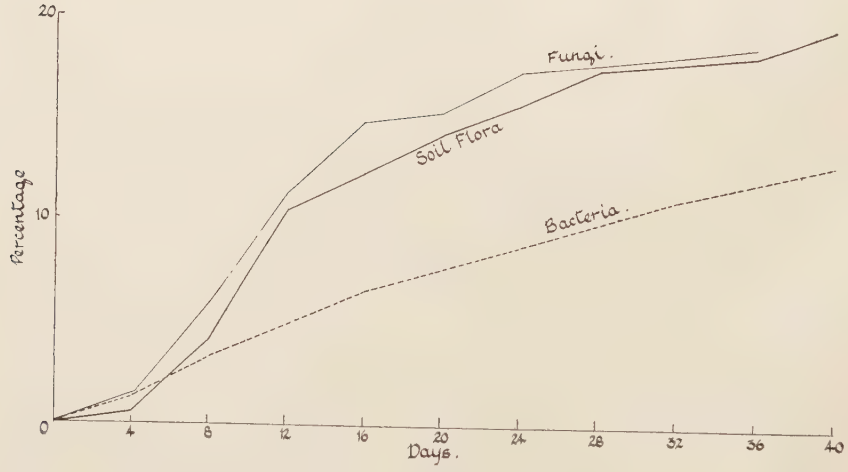
Series VII. Decomposition of rice-straw by bacteria.

This series was devoted to the study of bacterial activities in the decomposition of organic materials. As it was not possible to obtain all the bacteria from one kind of medium, the following media were used for their isolation: (1) Thornton's medium (37), (2) nutrient agar, (3) nitrate agar, (4) filter paper in mineral salt solution. As the micro-flora is shown to change (24) during different stages of decomposition of cellulosic materials, a mixture of fresh straw and decomposed manure was used as a source of these bacteria. The temperature of incubation was 35° C.

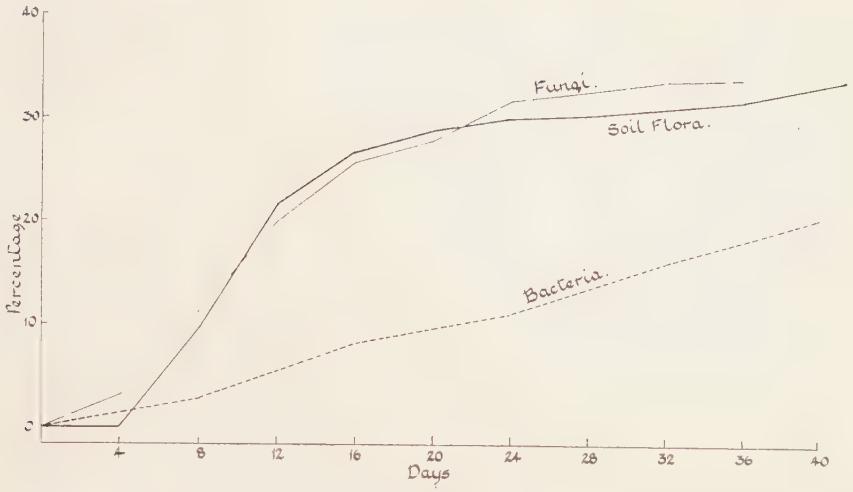
The same straw used in previous experiments was utilised in this case; but owing to the lack of sufficient quantity of this straw, the period for analysis was lengthened to 8 days. The methods of sterilisation and inoculation of this straw were the same as in Series VIII. Bacteria obtained on these media were used for inoculation without any attempt to identify them. In addition to the cellulose decomposing bacteria obtained on the filter paper medium, pure cultures of *Spirochaeta cytophaga* and *Microspira agar-liquefaciens* were also added. It was hoped that this mixture of bacteria would be a fair representation of all those active in the decomposition of organic materials. The decomposition in this case was very slow and the straw did not lose its tubular structure during the whole experimental period of 40 days.



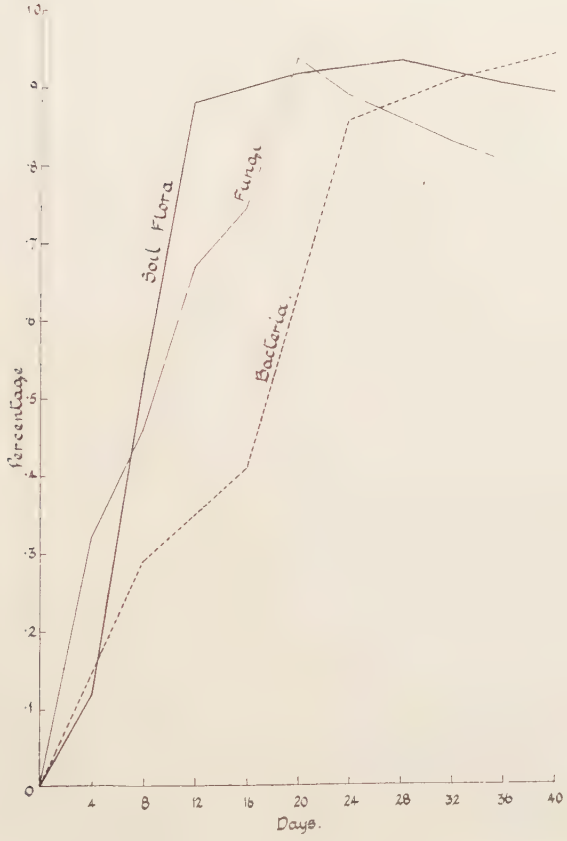
Graph 3. Loss of organic matter.



Graph 4. Loss of pentosans.



Graph 5. Loss of cellulose.



Graph 6. Nitrogen factor.

In all these series attention was specially directed to the quantitative estimation of the loss of organic matter, pentosans and cellulose. Incidentally the nitrogen-factor was also determined. The results are represented graphically.

Series VIII. Nitrification tests.

In this series, the products of the activities of these micro-organisms (Series VII, VIII and IX) were subjected to nitrification tests. A quantity of 200 gm. of soil was used for each bottle and these various materials in finely powdered condition were added in equal amounts of 40 parts of nitrogen to a million parts of soil and thoroughly mixed. Moisture was kept at 60 per cent. of the saturation. A control was run with the same amount of soil at the same moisture content. All the bottles were incubated in a cellar where the temperature was about 12° to 15° C. In some cases the experiment was repeated with the soil of low nitrate content.

After an incubation of one month, analysis for nitrate was carried out by the method described by Russell and Page (25) in their methods for soil analysis. The method depends upon the oxidation of organic matter in the water extract of the soil by alkaline permanganate and determination of ammonia obtained by reduction of nitrate with Devarda alloy in the residual liquid. The results are calculated as nitrate parts per million and are given in Table VIII.

Table VIII.

Nitrate nitrogen—parts per million. Experimental period—one month.

Rice-straw inoculated with	Control soil only	Rice-straw decomposed for 16 days only	Rice-straw decomposed for 36 days only
I. Soil extract (Series VII)	(1) 53·7	36·8	55·7
	(2) 19·1	13·1	20·1
II. Fungi (Series VIII)	(1) 53·7	52·9	55·6
	(2) 19·1	18·9	19·8
III. Bacteria (Series IX)	(1) —	—	—
	(2) 19·1	8·8	11·0

In the case of bacteria straw, which had been decomposed for 40 days, was used.

Discussion. It is quite evident from the results (see Graphs 3, 4, 5), that, given the proper conditions, the combined fungi are as active in the decomposition of the organic materials as the whole soil micro-flora. It must be admitted that the preliminary conditions in both cases were not exactly similar. Firstly in the case of fungi, the straw was subjected

to the drastic treatment of sterilisation. Chemically this was found to affect only the cellulose which gave a yield about 4 per cent. less than in the unsterilised straw. It would be impossible to judge what physical changes it had brought about; but it has been generally assumed that autoclaving makes a plant material more susceptible to microbial attack. Secondly inoculation in this case was far in excess of what would happen under natural conditions. The difference between the coefficients of curves for the loss of organic matter in the case of fungi and soil micro-flora respectively is not significant. The loss of pentosans as well as cellulose gives further confirmation as both the curves for fungi run more or less closely parallel with those of soil flora (see Graphs 4, 5). Therefore, even after making allowances for differences of treatment in the two cases, it can be fairly assumed that fungi alone can do the whole work of decomposition almost as efficiently as the soil micro-flora.

The bacteria, on the whole, do not seem to be as active as fungi. In both these cases the condition of the medium was exactly similar. Inoculation in this case was also far in excess of what occurs under ordinary conditions. Though it is not claimed that all the bacteria active in the soil were obtained, different types of media used for their isolation would assure a fair representation of the prominent bacteria. A similar limitation was also working in the case of fungi as only three species were used in the experiment. It is therefore justifiable to assume that fungi show a far greater activity than bacteria in the decomposition of organic matter, and therefore under natural conditions they play a more important part in the decomposition of cellulosic materials.

Though comparison of the nitrogen factor (see Graph 6) leads more or less to the above conclusion, there is a striking dissimilarity at later stages between the three curves. It would seem that although fungi during their vigorous growth locked up soluble nitrogen supplied as ammonium carbonate, they began to ammonify some portion so absorbed. The curves therefore showed a rapid downward tendency, and had the experiments been continued after 40 days, the nitrogen factor would probably have been greatly reduced. In the case of bacteria quite the reverse phenomenon was observed during the whole experimental period. The locking of nitrogen was very slow but rose during the whole experimental period. The curve for the soil micro-flora seemed to be a balance between these two curves and might indicate a balance between the activities of these two groups of micro-organisms.

The nitrification tests brought out two important points. Firstly, they showed that the loss of organic matter was no proper index of the

availability of nitrogen. Rice-straw had lost 45 per cent. of its organic matter during 36 days of its decomposition, but the nitrogen it had locked up during this process from the ammonium carbonate due to the activity of the micro-organisms did not seem to be easily nitrifiable. No doubt this decomposed material had no unfavourable effect on the nitrate already present in the soil, observed in the case of the straw which was decomposed only for 16 days. It could be assumed therefore that easily available carbohydrates which are generally considered to cause reduction in nitrates are broken down during those 36 days; but on the other hand the ammonium compound which was assimilated during this process by micro-organisms seemed to be transformed into some resistant complex which would be required to pass through some other stages of decomposition before being easily nitrified.

Secondly, fungi seemed to be more efficient in removing easily assimilable carbohydrates as the product after 16 days' decomposition in this case had not led to any loss of nitrate already present in the soil. The curves (see Graph 4) for the loss of pentosans indicated that fungi used more pentosans during the first 16 days than the total soil flora and this might be the reason of the big difference in the nitrate nitrogen figures in the two cases. The loss in the nitrate nitrogen in the case of bacteria even after 40 days could be easily explained by the slow decomposition the material had sustained during this period.

Series IX. Pot culture tests.

In this series, pot culture tests were carried out on *Coprinus* to see whether its nitrogen was easily available to plant growth. To get a comparative evaluation, ammonium sulphate and dried blood were also tried.

Coprinus was grown on straw mixed with assimilable nitrogen. As the temperature of the growth was 35° C. there was much vegetative growth, all the fruiting bodies being sterile. It was separated from straw, dried and powdered. This separation from straw was quite easy as the fruiting bodies rising above the surface of the straw could be easily removed, but in some cases bits of straw remained attached to the fungus, and it was therefore assumed that the material thus obtained contained about 2 per cent. straw. Its nitrogen content was found to be 3·5 per cent. of dry weight.

Four pots were used for each set and the material was added to the soil as 55 parts of nitrogen to one million parts of soil. Mustard was grown in pots as it responds well to nitrogen. *Coprinus* pots showed

very good growth at the start, but at later stages they lagged behind, and at the time of cutting there was no significant difference between this set and the control. The plants in both sets showed early maturity, and were thin with less leaf area. The results of the wet and dry weight of the plants are given in the following table:

Table IX.

Availability of nitrogen for plant growth.

Average for four pots in each set.

	Wet weight gm.	Dry weight gm.
Control	14·4	3·85
Ammonium sulphate	29·1	7·4
Dried blood	24·7	7·1
Coprinus	17·7	4·7

The results clearly show that the nitrogen in *Coprinus* is not in an easily available condition. This would very well explain the low nitrifiability of manures observed in Series VIII. During the 36 days period of the decomposition of the straw, the immobilised nitrogen may have been mostly in the fungus bodies.

C. *A study of the behaviour of certain fungi which were found to play the more important part in the process.*

It is quite clear from the previous section that fungi are important agents in the decomposition of plant materials in the presence of assimilable nitrogen. They can go through the whole process necessary to produce rotted organic manures which are found by the nitrification test to be as good as those ordinarily produced by the activity of all the groups of micro-organisms present in the soil. It is therefore of considerable importance to study their behaviour in pure culture towards various food constituents, and in this section an examination is made of three fungi active in such decomposition.

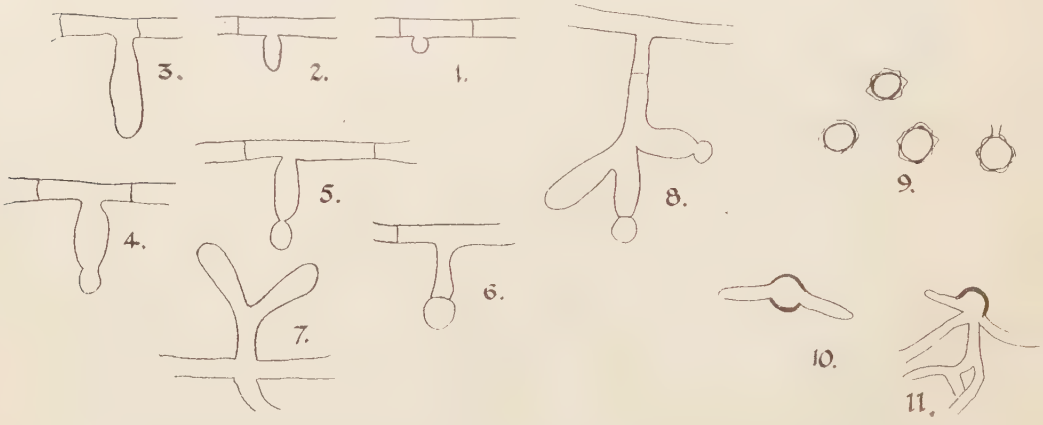
Method of Isolation.

Coons' agar acidified to $p\mathrm{H} = 4$ was used as a medium for isolating these fungi. The inoculum was prepared from a decomposing heap of wheat straw treated with assimilable compounds of nitrogen. Two temperatures were used for incubation, viz. 35° C. and 50° C., the temperature of the decomposing heap being about the latter. Fungal growth was obtained on the medium at both the temperatures and this was found to be a mixture of fungi: further separation was done on potato agar.

Morphological description.

Aspergillus sp. (resembles most closely *A. fumigatus*).

Colony on synthetic potato-agar (5), *p*H 6·2, temperature 35° C., white in vegetative stage, green when sporulating, turning brown with age; strict, with scattered aerial hyphae, secondary growth; no coloration of the medium. Submerged and superficial sterile hyphae creeping, greatly branched, septate, hyaline. Margin of the colony spreading, transparent. Conidiophores unbranched (rarely forked in two), aseptate, arising directly from the substratum or as side branches; when from the substratum, 80 to 200 × 6 to 10 μ, when as side branches 20 to 42 μ in length. The surface of the conidiophore is smooth, hyaline or scattered with granules. The conidiophore ends with a globose swelling, brownish in



Text-fig. 1. *Acremoniella* sp. (*velutina*?). 1–6. Development of spore. 7–8. Branched sporophores. 9. Mature spores. 10–11. Germination of spores. All magnified × 600.

colour, 10 to 22 μ in diameter, closely beset with simple sterigmata, pointing forwards, very close, numerous, narrow at the tip 6 to 8 μ in length. Conidia oval, 2 to 4 μ in diameter, smooth, hyaline singly, green when young, brown when mature in mass, in long chains, forming dark cylindrical heads 58 to 90 × 37 to 45 μ; chain of spores broken up readily when mounted. Germination of the spores commonly by two tubes at opposite ends of the spores.

Acremoniella sp. (resembles most closely *A. velutina* (Fuck) except in the shape of the spores).

Colony on synthetic potato-agar (5), *p*H 6·2, temperature 50° C., white in

vegetative stage, green turning to black when sporulating. Sectoring of the colony is very common. Strict with scattered aerial hyphae. No secondary growth. Coloration of the medium between cinnamon and fawn colour Ridgway (30). Margin consisting of submerged vegetative hyphae. Hyphae complexly branched, hyaline, septate; no differentiation between vegetative and reproductive hyphae. Surface mycelium carrying short sporophores laterally, generally single, rarely branched in twos and threes, hyaline, septate or aseptate; occasionally two or more arise at the same place or on opposite sides forming a cluster, 15 to 32 μ. Spores single on each sporophore, oval when young, globose when fully developed, brown, thick-walled, with markings on the surface, 6 to 9 μ. The spore breaks from the sporophore, sometimes retaining a portion of the latter. Germination normally by two or more tubes from a single opening. (See Text-fig. 1.)

Coprinus sp. (resembles most closely *C. fimetarius* Fr.).

The morphological examination in this case was carried out on straw moistened with assimilable nitrogen at laboratory temperature (about 15° C.).

Cap, when young ovate, 2 to 3 cm. high when fully grown, margin unequal, then more or less expanded, companulate, at first even; during growth the cuticle gets torn into adpressed shaggy scales which can easily be removed and are white in colour, interstices grey, disc pale ochraceous, remaining entire, margin torn; stem white, 3 to 5 cm. long at ordinary temperature (15° C.), more (10 to 15 cm.) at 35° C., cylindrical, sub-equal or slightly upwards, fibrous, hollow, with a cord of filaments in the cavity, covered with silky hairs which soon fall off, even, bulbous at the base, bulb solid, ring absent; gills free, distant from the stem, white then blackish; spores black, elliptical, 9 to 12 × 7 to 9 μ, germination by two or more tubes.

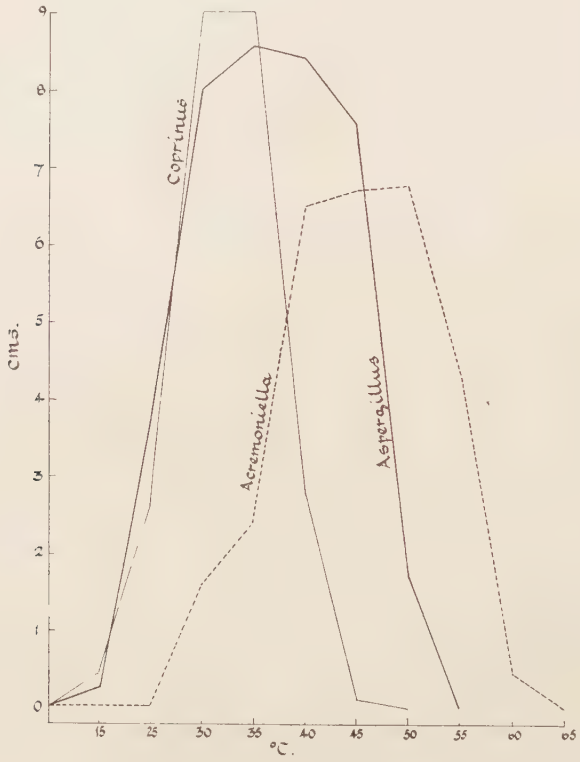
Physiological Studies.

1. *Temperature relationship.*

The growth of these fungi at various temperatures was studied on potato-agar in petri-dishes. Four petri-dishes, containing the same amount of medium (20 c.c.), were utilised for every temperature, and a definite quantity of inoculum of spores in sterile tap water (1 mm. loop) was placed in the centre of each petri-dish. They were then placed in columns face downwards in moist containers, a precaution taken to prevent drying of the medium at high temperatures. Generally the

incubation period was only for six days, but where the growth was slow it was extended to 12 and 24 days.

Measurements of the growth were made in every case on the 3rd, 6th, 12th and 24th day in two directions at right angles to each other, each measurement being made to the nearest half-millimetre. In cases where the colony did not grow circularly, measurements were made along the long and short diameter and the average taken. In the case of *Coprinus* sp. measurements were taken only on the 6th day.



Graph 7. Six days' growth of fungi at different temperatures.

The stock cultures which supplied the inocula were grown on synthetic potato-agar at 35° C. in the case of *Aspergillus* and *Acremoniella*. The *Coprinus* was grown on straw at ordinary temperature and the spores were collected on sterile cover-slips at the time of their shedding. Owing to the difficulty of obtaining a uniform suspension of spores as a result of their impermeability, especially in the case of *Aspergillus* sp., the inoculum

in each case was likely to differ in the number of spores. A count of 10 such inocula showed that the number of spores varies from 22 to 57. The actual measurement of growth in the petri-dishes showed that this big variation had no significant effect on the growth of the colony and therefore the quantitative measurements are sufficiently reliable. The results are represented in Graph 7.

2. *Growth on media containing carbon and nitrogen compounds.*

The mineral salt solution used as a basis for the study of the behaviour of these fungi towards different carbon and nitrogen compounds had the following composition:

$MgSO_4.7H_2O$	0·5 gm.
K_2HSO_4	1·0 ,,
KCl	0·5 ,,
$FeSO_4$	0·01 ,,
Water	1000 c.c.

Compounds of nitrogen were added at the rate of 2 gm. per 1000 c.c. of the medium and include peptone, ammonium sulphate, sodium nitrate and asparagin. The carbon compounds were used at the rate of 1 per cent. and consisted of dextrose, saccharose, maltose, xylose, arabinose, galactose, mannose, starch, straw gum, cellulose and lignin. All the media contained agar as 15 gm. per 1000 c.c.

Cellulose for the medium was obtained by dissolving filter paper in Schweitzer's reagent and precipitating it with acid (18). In addition to the cellulose agar medium, filter paper strips in mineral salt solution were also tried for the fungus growth. Lignin was extracted from wheat-straw by the method of Beckman, Liesche and Lehman (2).

The study of the behaviour of these fungi on the various media was done in petri-dishes. The amount of inoculum as well as the method of inoculation was the same as described in temperature relationship. The petri-dishes were incubated at the optimum temperature for the growth of these fungi, viz. 35° C. in the case of *Aspergillus* and *Coprinus* and 50° C. for *Acremoniella*. In the case of the latter, additional petri-dishes were incubated at 35° C. for certain media in which the fungus did not show good growth at the higher temperature. The period of incubation was generally 12 days.

The petri-dish cultures were made in order to take quantitative measurements of growth; but it was soon found that though this method was fairly accurate for one medium under different conditions, it was

Table X.

Behaviour of fungi on pure nitrogen and carbon compounds.

Period 12 dyas	35° C. *Aspergillus* sp.* Growth	35° C. *Aspergillus* sp.* Sporulation	50° C. *Acremoniella* sp. Growth	50° C. *Acremoniella* sp. Sporulation	50° C. *Acremoniella* sp. Colour of the medium	35° C. *Coprinus* sp.† Growth
Nitrate+(carbon compounds)						
Glucose	××××	××××	Growth in all cases poor, very little sporulation			××××
Saccharose	××××	××××				××
Maltose	××××	××××				××××
Xylose	××××	××××				×××
Arabinose	××××	×××				××
Galactose	××××	×××				×
Mannose	×××	×××				×
Starch	××××	××				××××
Cellulose	××	××××				Nil
Straw gum	××	×				—
Lignin	Nil	Nil				—
Peptone+(carbon compounds)						
Glucose	×××	×××	××××	××××	Nil	××××
Saccharose	×××	×××	××××	××××	Between cinnamon (XXIX) and fawn colour (XL)	×××
Maltose	—	—	××××	××××	Nil	××××
Xylose	××××	××××	××	/	,,	—
Arabinose	××××	××××	×	/	,,	—
Galactose	—	—	×××	××××	,,	×
Mannose	—	—	××××	××××	Same as saccharose	×
Starch	××××	××××	×××	×××	Nil	××××
Cellulose	—	—	Nil	Nil	,,	Nil
Straw gum	—	—	×××	×××	,,	××
Lignin	—	—	××	×××	,,	×
Ammonium sulphate+glucose	××	×	×	—	,,	—
Asparagin+glucose	×××	×××	××	×	,,	××
Casein+glucose	××××	××××	××××	×××	Varies between *i* and *k* (XXVIII)	×××
Egg-albumen+glucose	××××	××××	××××	××××	Cameo brown (XXVIII) *k*	××××
Potato agar	××××	××××	××××	××××	Same as saccharose	××××
Synthetic potato agar	×××	××	××	×	Nil	×××
Straw extract agar	×××	××	×××	××	,,	—
Prune extract agar	××××	××××	/	Nil	,,	/
Filter paper in mineral salt	××	××××	Nil	,,	,,	Nil
Raulin's solution	××××	××	,,	,,	,,	××××
White of egg (liquefying power)	×× Nil	××××	××/	××	Colour same as egg albumen agar. No liquefaction	×××× Nil

* *Aspergillus*, no colour in the medium. † *Coprinus*, no colour in the medium, no sporulation.

N.B. Period of incubation—12 days. Ridgway's *Colour Standards and Nomenclature.*

The symbol ×××× represents the best growth as well as the best sporulation, while the symbol / represents germination in the case of the growth and visibility of the spores under the microscope in the case of sporulation. The others represent the intermediate stages.

quite unsuitable for different media owing to the variation in the type of growth of the one fungus. By this system thin spreading growth gives high quantitative results even though the visual inspection would show quite the reverse. Also it does not take sporulation into account. For these reasons quantitative measurements were discontinued in all these cases. A qualitative idea is obtained by the visual inspection of

the growth on three petri-dishes in each case. This is symbolically represented in Table X. The symbol $\times\times\times\times$ represents the best growth and the symbol / only the germination of spores, the others representing intermediate stages. The symbol / in the case of sporulation indicates that the spores are visible under the microscope only.

In addition to the above media, the following standard media were also tried: prune extract agar, straw extract agar, Raulin's solution, synthetic potato agar (5), casein glucose agar, potato agar, egg-albumen glucose agar. The liquefaction power of these fungi was tested on white-of-egg. The usual procedure of utilising gelatine for such an experiment could not be adopted owing to the high temperature necessary for this study.

3. *Study of enzymic activity.*

A few tests of the enzymic activity of these fungi were carried out according to Crabill and Reed's methods (6), and the results are shown in Table XI.

Nitrogen was added to the stock medium in the form of nitrate and peptone. As can be seen from Table X, while nitrate is favourable for the growth of *Aspergillus* and *Coprinus* it is inhibitive to the growth of *Acremoniella*. On the other hand *Aspergillus* prefers nitrate to peptone which is most suitable for the growth of *Acremoniella*. Thus for the study of each enzyme it was necessary to use two media varying in the type of nitrogenous compounds.

4. *Action on cellulosic materials.*

Rice-straw was chosen as a cellulosic material for this study. It was sterilised in bottles and inoculated with the pure cultures of these fungi singly as well as in all possible combinations. Method of sterilisation, addition of ammonium carbonate and inoculation of these fungi was exactly the same as described in Section B, Series II.

All the bottles were inoculated at 35° C. In the case of *Acremoniella* an additional bottle was incubated at 50° C. which is the optimum temperature for its activity. The experiment was stopped after 36 days and the contents were analysed. The results are given in Table XII.

Acremoniella did not show any activity at either temperature, while *Aspergillus* took nearly a fortnight for a start. In the case of the latter, sporulation was the prominent feature. Though *Coprinus* showed early growth, the decomposition was very slow even in this case. The various combinations of these fungi in twos worked better than the individual fungus; but all three together seemed best for the decomposition.

Table XI.

Enzymic activity of fungi.

Enzymes	Medium	Growth	Dissolution of the particles	Halo	Reaction
		Aspergillus sp.			
Erepsin	Casein agar	Fair	Slight	Fair	—
Trypsin	Egg-albumen agar	„	Good	None	—
Amidase	Asparagin-rosolic acid agar	Good	—	—	Deep brilliant red, widely diffused
Cytase	Straw gum agar	Fair	Doubtful	None	—
Cellulase	Cellulose agar	Good	None	„	—
Amylase	Starch agar	Very good	—	—	Yellow
Lignin-decomposing enzyme	Lignin agar	None	None	None	—
		Acremoniella sp.			
Erepsin	Casein agar	Poor	Fair	None	—
Trypsin	Egg-albumen agar	Good	Good	„	—
Amidase	Asparagin-rosolic acid agar	Fair	—	—	Slight red
Cytase	Straw gum agar	Good	Slight	None	—
Cellulase	Cellulose agar	None	None	„	—
Amylase	Starch agar	Very good	—	—	—
Lignin-decomposing enzyme	Lignin agar	Fair	Doubtful	None	—
		Coprinus sp.			
Erepsin	Casein agar	Good	Good	None	—
Trypsin	Egg-albumen agar	Very good	Very good	„	—
Amidase	Asparagin-rosolic acid agar	Good	—	—	Deep red diffused
Cytase	Straw gum agar	Slight	Doubtful	None	—
Cellulase	Cellulose agar	None	None	„	—
Amylase	Starch agar	Very good	—	—	Yellow
Lignin-decomposing enzyme	Lignin agar	Slight	Doubtful	None	—

Table XII.

Decomposition of rice-straw by fungi.

Calculated on 100 gm. of dry matter.

	Loss of dry matter	Nitrogen factor
Coprinus	23·2	0·5
Coprinus + *Aspergillus*	27·2	0·51
Coprinus + *Acremoniella*	26·3	0·43
Aspergillus	16·0	0·54
Aspergillus + *Acremoniella*	28·0	0·34
Acremoniella	0·0	—
Aspergillus + *Acremoniella* + *Coprinus*	40·6	0·76

5. *Physiological characteristics of individual fungus.*

Aspergillus sp.

The optimum temperature for the *Aspergillus* seems to lie between 30° and 40° C. and the maximum at about 50° C. Its growth below 30° C. is very slow and at the ordinary temperature of the laboratory, which was about 15° C., the spores took about 12 days to germinate. Thus this fungus cannot be active in the decomposition of cellulosic materials at a temperature below 30° C.

Table X indicates that the *Aspergillus* has no special affinity for any one carbohydrate. Though there is a slight variation of growth on the different media, it is not significant to require special consideration. It shows moderately good growth even on cellulose media. On the other hand it shows poor growth on straw gum, which cannot therefore act as a substitute for its hydrolytic products—xylose and others. This might be the reason for the slow activity of the fungus on rice-straw, as the lack of this hydrolysing enzyme would naturally prevent it from making use of the easily available food material—the pentosans.

Among the nitrogen compounds, nitrate comes the first, though in some carbohydrate media, peptone is as good as nitrate. Asparagin comes next, but ammonium sulphate supplies poor nourishment. As this fungus shows the best growth on an acid medium (pH = 4·6) this inhibitory effect of ammonium sulphate may not be due to acidity.

The colour of the spores seems to be dependent upon the nature of the carbohydrates. While dextrose and saccharose cause the production of dark green spores, the fungus on arabinose and starch has spores of a paler tinge. Further, the browning of the spores observed in the case of growths on potato agar about the 6th day, was not visible in the case of growths on all these carbohydrates even on the 12th day. A study of Table XI indicates that the predominance of sporulation over the vegetative stage is due to the poverty of the medium in the requisite food ingredients. Cellulose agar or white-of-egg are good examples. On the other hand, rich media such as Raulin's solution brings about quite the reverse effect.

Acremoniella sp.

The optimum temperature for the growth of *Acremoniella* lies between 40° and 50° C. and the maximum at about 60° C. Though isolated spores do not germinate at higher temperatures, they are found to germinate up to about 70° C. if inoculated in agglutinated mass. The spores are not found to germinate at all below 25° C. The necessity of

high temperature for its growth indicates that ordinarily this organism cannot be active in the decomposition of cellulosic materials. But during the first stages of the decomposition of manure heaps the temperature goes as high as 55° C. and sometimes even higher. Generally speaking thermophyllic bacteria are considered responsible for the decomposition during this period; but it seems reasonable to assume that this fungus can also take part during that process.

Though the fungus requires high temperature for its growth, it seems to be susceptible to sudden changes of temperature. Thus it is found that an inoculum from cultures grown at 50° C. and kept afterwards at room temperature for more than a month will not grow if incubated at its optimum temperature of 50° C. It is necessary either to raise it gradually from lower temperatures (35° C.) upwards or to inoculate the spores in agglutinated mass if incubated at once at the high temperature. Even in the latter case one or two transfers are necessary for it to regain its original vitality. Whether it would show the same phenomenon under the natural condition of its activity is not known. But this characteristic is repeatedly observed on artificial media. Sectoring occurs frequently in cultures of this fungus and is probably the basis of its change in temperature relationships.

This fungus shows great diversity in growth on different nitrogen and carbon compounds. Nitrate and ammonium sulphate have an inhibitory effect. Not only the growth is poor, but the hyphae are closely felted together and in some cases as in nitrate saccharose agar, they showed beaded appearance. Sporulation is also generally absent. In some cases sporophores can be seen under the microscope with immature spores. Peptone is the best nitrogenous food, asparagin coming next to it.

Among the carbon compounds, pentoses seem to supply poor nutrients to this fungus; but it grows very well on straw gum. Perhaps the concentration of these sugars might be too high for it. In the case of straw gum, insolubility of the gum would not lead to the increase in the concentration. It does not show any growth at all on cellulose in solid as well as liquid media. On lignin some growth is made though the fungus is not vigorous on it. In other carbohydrates, *Acremoniella* shows vigorous growth and in such cases, sectoring is common.

The general colour of the colony is grey though individual spores are dark brown. The spores, in contrast to those of *Aspergillus*, remain moist in all the media tested. They are not therefore easily scattered by the wind as is the case with the *Aspergillus* spores. *Acremoniella*

also produces colour in the medium which is in certain cases, as on egg medium, very characteristic.

Coprinus sp.

The optimum temperature for the growth of *Coprinus* lies between 30° and 35° C. and the maximum at about 45° C. In this case, the best temperature for growth is not the best for spore production as almost all the fruiting bodies are sterile. The fruiting bodies grow very vigorously, reaching a height of 8 inches on straw and also branch copiously; but the cap is almost always very small, hard and yellow, without scales, and sterile. At this temperature only in rare cases was it found to give fertile fruiting bodies. But sclerotia—10 to 15 brown irregularly shaped bodies—are quite common both on the media as well as on the straw. At ordinary temperature as well as at 25° C. growth is very slow, the maximum height to which the fruiting body is found to reach being 3 inches. But generally all the fruiting bodies are fertile. The shedded spores kept on sterile cover-slips are found to germinate even after six months.

Except galactose and mannose, all the carbohydrates are suited to its growth. The most vigorous growth is found on glucose and maltose, where the colony not only spreads even outside the petri-dish due to the fluffy growth, but is full of sclerotia.

V. General Discussion and Conclusions.

In the discussion of this work, it must be borne in mind that plant materials containing large quantities of hexoses and starches are generally used as food either for animal or man and are not therefore likely to be utilised as waste for conversion into manure. Thus the materials available for manurial purposes are deficient in both these constituents. However, the process of rotting does not appear to be unfavourably influenced by the elimination of these constituents and it can therefore be assumed that they have no important bearing on the decomposition of these materials.

As regards cellulose, this forms a major portion of the fundamental plant tissue and is of importance in the decomposition. Perhaps the greatest difficulty in tracing the behaviour of cellulose is the lack of any reliable method for its estimation. Out of all the methods so far proposed, the chlorination method of Cross and Bevan (8) is generally considered to be the best; even by this method, however, not only does the preliminary alkali treatment lead to a slight loss of cellulose, but the

chlorine after first combining with lignin starts to attack the cellulose itself. As the decomposition by organisms proceeds, therefore, the surface area of the cellulose is increased which makes it more susceptible to the chemical treatment. This is confirmed by Graph 5 which shows that the loss of cellulose from the rice-straw decomposed by fungi alone is very rapid though only one of these fungi is found to grow slightly on pure cellulose. The apparent large loss of cellulose is therefore not purely due to the microbial activity and would not give an index as to its food value to the micro-organisms. This must be borne in mind in seeking to interpret any analytical data in connection with cellulose decomposition. Thus failing any reliable direct evidence it is necessary in order to obtain an idea of the importance of cellulose to use indirect means. This has been made possible by the physiological studies of the organisms most active in the rotting of this type. An investigation into the relative importance of bacteria and fungi in these decompositions shows the latter to be far more active than the former. The behaviour of these active fungi towards different purified carbon compounds shows that pure cellulose is itself a poor nourishment especially at the start, and it seems, therefore, that though cellulose may be easily decomposed once the organisms are active, it would not be a factor controlling the "decomposibility" of the material.

Investigations by many workers have proved that lignin is very resistant to microbial attack. Except certain timber rotting fungi such as *Trametes pini*, etc., which do not commonly occur either in soil or manure, organisms do not flourish solely on lignin. The physiological studies here reported of certain fungi acting in such decompositions also show that, except in one doubtful case ,lignin does not sustain their growth, and it is therefore quite natural to assume that lignin would not only be a poor nourishment to micro-organisms, but being a part of the fundamental tissue would, on account of its resistant nature, act as a physical barrier, thus hindering their ability to get their food. If this be true the high lignin content of any cellulosic matter would be detrimental to its decomposition and this is found to be the case with many of the materials.

Next to cellulose, hemi-cellulose forms an important constituent of plant tissue; but like cellulose, this is not a chemical entity as on hydrolysis it not only yields separate distinct compounds, but these compounds vary in quantity in different cellulosic materials. Thus its estimation, as a whole, does not lead one to any definite conclusions. The only possible way, therefore, of getting an idea of the value of these

various products of hydrolysis in the process of decomposition is to study their effect on the activity of micro-organisms. It has been shown (Series IV) that both galactose and mannose offer poor nourishment to the organisms active in such decompositions. The physiological studies of fungi also confirm their poverty as nourishment to the most active fungus (*Coprinus*). On the other hand, both by its rapid disappearance during the process of decomposition, as well as by the nutritive value of the products of hydrolysis, the pentosan part of the hemi-celluloses is found to supply the best energy to these micro-organisms. Pentosans form the larger part of the material which is attacked at the start, and though the loss in other constituents at later stages masks their importance, they are found to be essential to start the process of decomposition. The predominance of fungi in such decompositions further supports this assumption as studies on their physiology show that pentosans either in their natural state or in hydrolysed form supply the best nutrition to these organisms. This also lends confirmation to the conclusions of various workers who consider pentosans as the most easily decomposable constituent.

The study of the decomposition of the carbonaceous constituents of plant materials leads one, therefore, to conclude that pentosans form the important microbial food, and they may thus be assumed to control the "decomposibility" of a material. Further investigation on varied materials to test the validity of this hypothesis resulted in the knowledge of the unreliable nature of the analytical method for the determination of pentosans. It was found that the Krober and Tollens (1) method for pentosan determination included also some part of cellulose which was found both by direct fermentation tests as well as by indirect analytical estimation to be resistant to microbial attack. The apparent large figure for pentosans observed in the case of certain materials, such as poplar wood, consisted largely of this resistant compound and was therefore very misleading. The method had therefore to be modified to get as far as possible an accurate figure for pentosans. This was achieved by deducting the furfuroids yielded by cellulose from the total furfuroids, considering the remainder as representing pentosans. Even in this case certain exceptions to this hypothesis were found, thus showing that there was some other constituent besides pentosans which had a bearing on the process of decomposition. As discussed above, lignin could be taken as such a compound. Since it is of resistant nature, it would be expected to act as a physical barrier to the microbial search for food materials and therefore a balance between the two factors—

nutritive or chemical and physical—would be likely to control the process of rotting. The application of this modified hypothesis in evaluating the results obtained (Table V) proved its validity; we are thus enabled to predict the "decomposibility" of a material by purely chemical analysis.

It is evident that the important condition for rapid decomposition of a material is the predominance of pentosans over lignin. This is found generally to be the case with the easily decomposable materials such as straws. On the other hand, woods consist of a large amount of lignin and are found to be very resistant. This indication of the basic principles underlying the process of decomposition naturally leads to the next step for its practical application, viz. the artificial stimulation of the process. There seemed to be two possible ways by which this could be achieved, either by a reduction of the lignin content or by an increase in the amount of pentosans. In the former case, it is essential to get an organism with special predilections for lignin; but among the micro-organisms active in such decompositions none is so far found to show such specificity. The second alternative was also found impracticable as organisms getting both their nitrogenous and carbonaceous food outside the tissues produced less decomposition of plant materials than was normally possible.

As regards the relative importance of the two groups of micro-organisms active in such processes, the present investigation proves that fungi are more important than bacteria, especially during the early stages of decomposition. Further, fungi are found capable of carrying out the whole process of breaking down of the cellulosic material as efficiently as the total soil micro-flora. But it seems that the nitrogen which these fungi immobilise in their body protein is not easily available for plant growth (see Series IX). It is therefore necessary that these bodies in turn must be decomposed by other organisms and the disappearance of fungal hyphae at later stages of decomposition suggests that nitrogen passes through the bodies of various organisms before it can be easily nitrifiable for plant growth.

In addition to the physiological peculiarities noted above, the study of these fungi shows a further interesting point. All three are most active at temperatures far above those usual for fungal growth and it would be an important point to note whether they themselves can bring about the rise in temperature necessary for their activity. A preliminary experiment in thermos flasks, when daily temperature records were taken of the straw which was *sterilised* and inoculated, very strongly suggests

this possibility, but further and more elaborate investigation is necessary before any definite conclusions can be carried out.

VI. Summary.

In mature plant materials pentosans form the most important food for micro-organisms.

The Klober and Tollens method for the determination of pentosans is not specific for these compounds.

While pentosans are easily attacked by micro-organisms, the other furfurol-yielding compounds are found to be resistant, and it is therefore essential to get a correct figure for pentosans. A possible method is suggested: to determine the furfuroids in the cellulose obtained by the chlorination method and to deduct this amount from the total furfuroids.

Two factors appear to control the decomposition of ripe cellulosic materials in the presence of assimilable nitrogen. The one is the food, or, better termed, *energy factor* which is the pentosans, the other is the physical or *inhibitory factor* which is the lignin. It is found that if the ratio of energy factor to inhibitory factor is above 1, the material is easily decomposed; but if it is below 0·5, the material is very resistant to microbial attack. The prediction of the "decomposability" of a material is thus possible.

Attempts to increase this ratio in resistant materials by the addition of carbohydrates proved unsuccessful. It was concluded that since micro-organisms obtained their food materials outside the tissues, they did not attack the tissues until the more easily available food-stuffs were exhausted. Thus the decomposition of the material was actually less than was possible under natural conditions.

Mannose and galactose do not appear to form suitable food for the micro-organisms concerned in these processes and it is concluded that the pentosan part of the hemi-celluloses is most important as microbial food.

The study of the relative importance of bacteria and fungi proves that under the conditions of these experiments, fungi play a more prominent part especially during the early stages of such decomposition.

The study on the availability of the nitrogen of the fungal bodies proves it to be of the resistant type. It seems that at later stages of decomposition under natural conditions fungi are decomposed by other organisms.

The ability of certain fungi isolated from such decomposing heaps, to grow at high temperature as well as on purified carbon constituents

of plants, and also the presence of almost all the enzymes necessary to hydrolyse the complex carbon constituents, further confirm their importance. The possibility of their activity under natural conditions in manure heaps is strongly suggested.

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EXPLANATION OF PLATES I—IV.

PLATE I.

Fig. 1. *Coprinus* sp. (*fimetarius*?) growing on sterilised oat-straw at laboratory temperature. Fertile sporophores are formed.

Fig. 2. *Coprinus* sp. (*fimetarius*?) growing on sterilised rice-straw at 35° C. Sporophores are formed which frequently push the cotton-wool plug out of the neck of the bottle and emerge several inches. The sporophores are aborted and sterile.

PLATE II.

Fig. 1. *Acremoniella* sp. (*velutina*?). Six days' growth on Czapek's agar. At 25° C. and 65° C. the spores have germinated but are not visible in the photograph.

Fig. 2. *Aspergillus* sp. (*fumigatus*?). Six days' growth on Czapek's agar. At 15° C. and 55° C. the spores have germinated but are not visible in the photograph.

Fig. 3. *Coprinus* sp. (*fimetarius*?). Six days' growth on Czapek's agar. At 15° C. and 45° C. the growth is just visible. At 10° C. and 50° C. germination occurs but no growth is visible in the photograph.

PLATE III.

Fig. 1. *Acremoniella* sp. (*velutina*?). Growth on peptone medium + arabinose.
Fig. 2. „ „ „ „ + xylose.
Fig. 3. „ „ „ „ + mannose.
Fig. 4. „ „ „ „ + maltose
Fig. 5. „ „ „ „ + saccharose.
Fig. 6. „ „ „ „ + lignin.
Fig. 7. „ „ „ „ + straw gum.
Fig. 8. „ „ „ casein medium.
Fig. 9. „ „ „ egg medium.

Fig. 3 shows sectoring of the colony.

PLATE IV.

Fig. 1. *Aspergillus* sp. (*fumigatus*?). Growth on glucose medium + nitrate.
Fig. 2. „ „ „ „ + peptone.
Fig. 3. „ „ „ „ + ammonia.
Fig. 4. „ „ „ „ + asparagin.
Fig. 5. *Acremoniella* sp. (*velutina*?). „ „ + nitrate.
Fig. 6. „ „ „ „ + peptone.
Fig. 7. „ „ „ „ + ammonia.
Fig. 8. „ „ „ „ + asparagin.

(*Received July 19th*, 1926.)

Fig. 1.

Fig. 2.

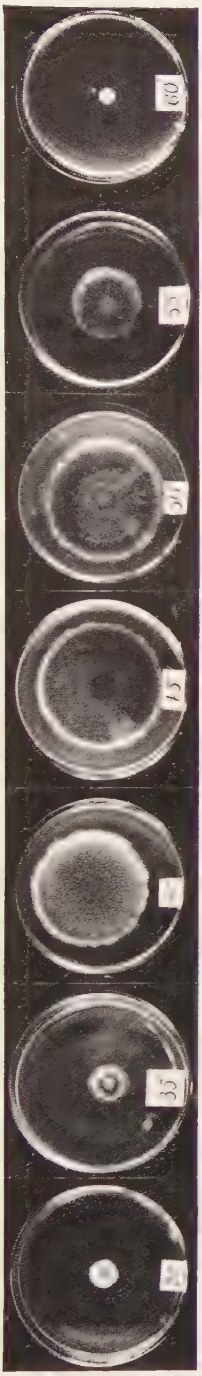


Fig. 1.

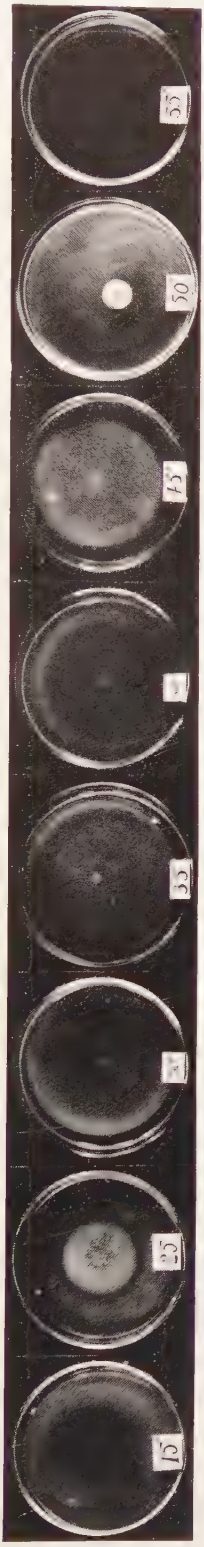


Fig. 2.

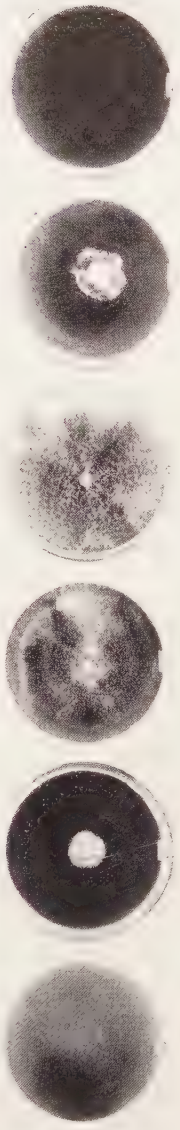


Fig. 3.

REGE.—Bio-chemical Decomposition of Cellulosic Materials (pp. 1–44).

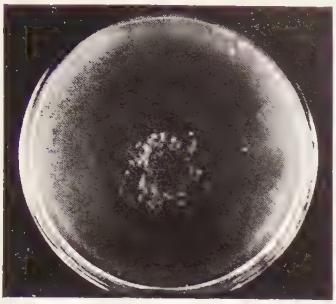


Fig. 8.

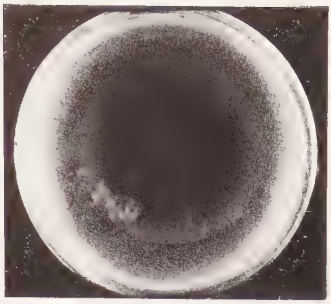


Fig. 9.

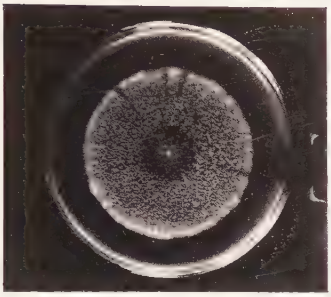
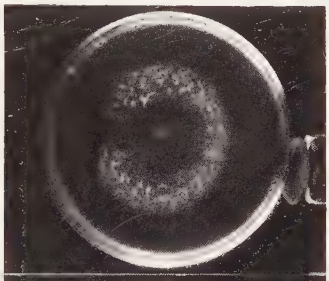


Fig. 7.

Fig. 3.



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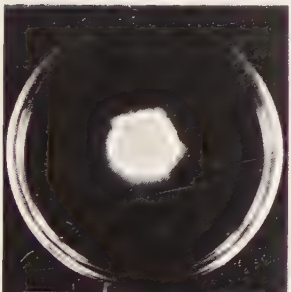

Fig. 2.

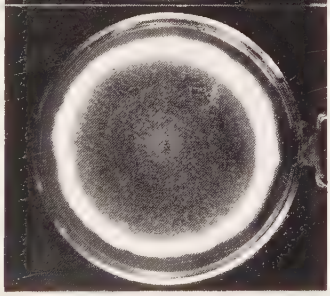

Fig. 5.

Fig. 1.

Fig. 4.

REGE.—Bio-chemical Decomposition of Cellulosic Materials (pp. 1–44).

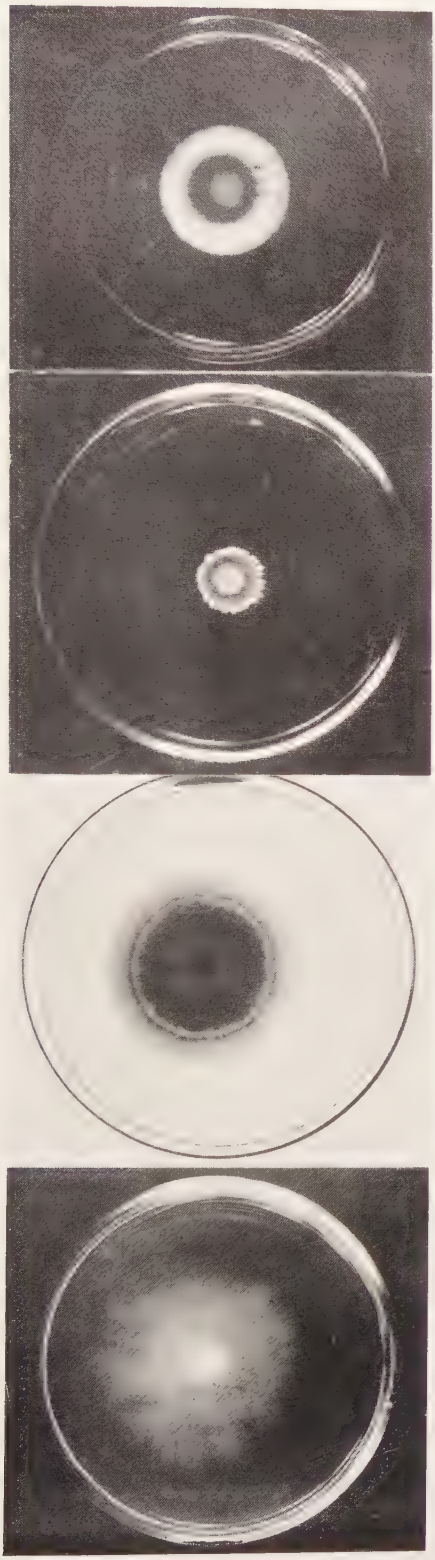

Fig. 1. Fig. 2. Fig. 3. Fig. 4.

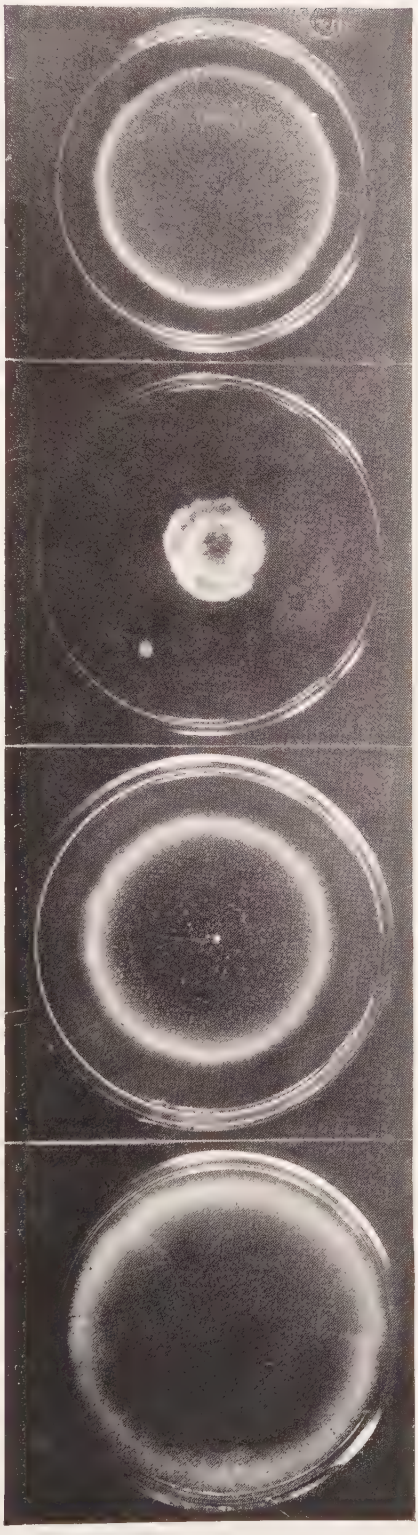

Fig. 5. Fig. 6. Fig. 7. Fig. 8.

REGE.—Bio-chemical Decomposition of Cellulosic Materials (pp. 1–44).

THE INTER-RELATION BETWEEN SILICON AND OTHER ELEMENTS IN PLANT NUTRITION

By WINIFRED E. BRENCHLEY, D.Sc., E. J. MASKELL, Ph.D.[1], and KATHERINE WARINGTON, M.Sc.

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(With Plate V and 3 Text-figures.)

CONTENTS.

A. Introduction.

Silicon ranks amongst the elements that are usually found in the ash of plants, and numerous analyses are available to testify to its almost universal occurrence. Wicke (18) found sufficient silicon in beech

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bark to leave a skeleton when properly treated, lesser quantities being detected in other Cupuliferae, in species of *Acer*, and in many members of Urticaceae and Artocarpeae. Hattensaur (7) determined in *Molinia caerulea* 28·656 per cent. SiO_2 in the ash, being 0·646 per cent. of the whole plant, while Ott (13) found 70·64 per cent. SiO_2 in the ash of *Equisetum Telematia* and 41·73 per cent. SiO_2 in the ash of *Equisetum arvense*. In some cases the silicon content is very heavy, as in *Moquilea* sp. (fam. *Chrysobalaneae*), in which Crüger (1) found that the bark contained 30 per cent. ash, of which 96 per cent. was silica. As a general rule silica gradually accumulates with time in the ash, and in young organs the merest traces may occur in plants which, when mature, contain a heavy percentage. The most comprehensive information, however, is given by Wolff (19), who summarises the results of numerous analyses, giving the range and average percentages of SiO_2 in the ash of many plants. A few of the most typical may usefully be quoted here, to indicate the large amounts of silicon that are normally widely distributed through the vegetable kingdom.

Percentage of SiO_2 in ash.

Plant	Number of analyses	Average	Range
Meadow hay	106	28·73	63·2–10·4
Red clover	113	2·69	20·2– 0·0
Lucerne	12	9·54	27·9– 0·8
Barley (grain)	57	25·91	36·7– 3·7
„ (straw)	30	51·00	68·5–32·1
Wheat (grain)	110	1·96	5·9– 0·0
„ (straw)	18	67·50	72·5–49·6
Peas	40	0·91	3·0– 0·0
Potato tuber	59	2·04	8·1– 0·0
Sugar beet (root)	149	2·28	12·1– 0·0
„ (shoot)	25	10·17	33·5– 0·0

This very general presence of silicon led to the belief that the element was an essential nutrient for many plants, especially cereals, but it has been shown that even such plants which usually contain large quantities of silicon can come to perfect maturity in its absence. The idea also arose that the presence of much silica prevented the lodging of wheat, but as early as 1866 Pierre (15) showed this to be a fallacy, as varieties that contain most silica are often the most liable to lodge, as the bulk of the silica is accumulated in the leaves and the least in the nodes. For equal weights leaves contain 7 or 8 times as much silica as the nodes, and

Pierre suggested that if the leaves are removed before the ears ripen, lodging can often be prevented, although much of the silica in the straw is thereby removed. Pfeffer (14) supported the theory that the laying of crops after heavy rain is not due to the absence of silica, but rather to the partial etiolation of the basal portions of thickly sown plants.

It is interesting to note that the silicon content of the ash is not necessarily determined by the amount of silica available in the substratum. Richardson (16) claims that dune plants grown on almost clear silicate obtain and concentrate in their tissues the same mineral constituents, in approximately the same relative proportion, as the same species grown on ordinary soil, with apparently no extra storage of silica.

The function of silicon in plant nutrition has attracted much attention, both from the theoretical and practical aspects. Fliche (5 suggested a possible association between silicon and phosphorus, showing that chalk-avoiding plants like *Calluna vulgaris*, which had about 27 per cent. SiO_2 in the ash, also had about 10 per cent. phosphoric acid. Hall and Morison (6), after due consideration of the results obtained on certain Rothamsted plots and from special series of water cultures, concluded that the increased and earlier grain formation observed in the presence of silica, is due to an increased assimilation of phosphoric acid within the plant brought about by the silica. They do not cite any evidence that the function of phosphorus is in any degree usurped by silicon, or that phosphoric acid can be replaced by silicates as manurial constituents. Jennings (8) found an increase of 17·8 to 29·2 per cent. in dry weight in wheat seedlings grown with 1 per cent. silica added to the nutrient solution, and also an increase in the silica content of the plants[1]. Lemmermann and Wiessmann (9) stated that definite increase in yield was induced by silicon in presence of insufficient phosphoric acid, and a less increase if potash was deficient, the best results being obtained with colloidal silicate, this being attributed to an influence exerted on the plants and not on the soil. Later (Lemmermann, Wiessmann and Sammett (10)) this view was somewhat modified, and it was further stated that silica does not replace phosphoric acid in the nutrition of the plant, but indirectly increases the amount of phosphoric acid which can be taken up from the soil and the efficiency with which it is utilised. Nanji and Shaw (12), however, controvert this hypothesis, claiming that if phosphoric acid be absent but an abundant supply of silica be available, the latter is able to replace the phosphate

[1] Schollenberger (17) also obtained increased growth of various crops by the use of various silicate compounds in soil, both with and without the addition of other fertilisers.

without any detriment to growth; further, that conditions that are favourable for the assimilation of silica may be unfavourable to or even suppress the intake of phosphoric acid.

Densch (2) anticipated Lemmermann's results, finding that silica does not replace phosphoric acid, but that soluble silicate induces a stronger growth and greater intake of plant food constituents from the soil. This was further corroborated by Gile and Smith (3), who also found that silica gel greatly benefited the growth of plants receiving rock phosphate, presumably by increasing the quantity of phosphoric acid in solution, though little increase was brought about if acid phosphate were used.

In view of the possibilities of reducing the necessary quantities of phosphate or potassic fertilisers by means of silica compounds suggested by the work of the above investigators and others, experiments have been undertaken at Rothamsted to determine whether the use of silicates, supplementary to other forms of fertilisers, might be an economic proposition.

B. Relation between Silicon and Phosphorus in Plant Nutrition.

With a view to obtaining more exact information as to the possible replacement of phosphorus by silicon in the economy of the plant, the water culture method was employed, enabling silicon to be entirely excluded from the nutritive medium when necessary. The culture bottles were lined with purified paraffin wax to prevent solution of silicate from the glass, and pure analytical salts were utilised for preparing the nutrient solutions. Barley was grown in the presence and absence of phosphate, with or without the addition of soluble sodium silicate. The unit of silicate employed was that providing as much silicon per litre of nutrient solution as is equivalent, atom for atom, to the phosphorus normally supplied in the same amount of solution.

I. *Experiment* 1, 1924.

Two types of nutrient solution were employed, of *p*H 6·2 and *p*H 3·7, the only difference being the replacement of the acid potassium phosphate in the latter solution by a mixture of acid and alkaline phosphate in the former to change the *p*H value. Two amounts of silicate were utilised, providing Si $\equiv \frac{1}{8}P$ and Si $\equiv 1P$, and as the addition of these caused very considerable fluctuations in *p*H values, a second parallel series was grown in which the correction to the original *p*H was made

by means of standard HCl or NaOH. In this way it was hoped to eliminate variations caused by the varying acidity, and thus to concentrate attention on the effect of the silicate. It was ultimately found that the variations due to fluctuating *p*H values for the same treatment were very considerable, and for the sake of clearness only that series will here be considered in which the acidity was restored to its original *p*H value after the addition of silicate. The culture solutions were renewed as required, with five changes in all, the intervals between changes becoming shorter as growth went on. As the bottles were of 600 c.c. capacity, each plant thus had access to 3·0 litres of solution altogether. The *p*H value of the old solution in each bottle was determined at every change. The composition of the four solutions was as follows:

	With phosphate		Without phosphate	
	A (*p*H 6·2)	B (*p*H 3·7)	From A	From B
	gm.	gm.	gm.	gm.
Potassium nitrate	1·0	1·0	1·0	1·0
,, hydrogen phosphate	0·3	0·5	—	—
,, phosphate (alkaline)	0·27	—	—	—
Magnesium sulphate	0·5	0·5	0·5	0·5
Calcium sulphate	0·5	0·5	0·5	0·5
Sodium chloride	0·5	0·5	0·5	0·5
Potassium chloride	—	—	0·4	0·27
Ferric chloride	0·04	0·04	0·04	0·04
Distilled H_2O to make up 1 litre				

Barley var. Goldthorpe. Graded: ·06–·07 gm. Sown: March 3rd, 1924. Put in solution: March 11th, 1924. Harvested: June 11th, 1924.

a. Phosphorus present.

Good growth was made in both solutions, though the plants in the more acid one were distinctly more flaccid and less upright, shorter and lighter in weight than the others.

With *p*H 6·2 solution some increase in height and in green and dry weights occurred with silicate, but the differences were not well marked and probably were of little significance. The proportion of shoot to root, however, was considerably raised by the heavier dose.

With *p*H 3·7 solution silicate induced a marked increase in height and green weight, but the difference was less marked in the dry weight and shoot/root ratio.

b. Phosphorus absent.

Growth throughout was very poor, only one shoot being formed per plant except with the heavier silicate in the *p*H 6·2 solution, where an

Table I.

Barley data at harvesting.

Average of five plants in each set.

Treatment	Height cm.	Number of tillers*	Dry weight: Shoot gm.	Root gm.	Total gm.	Shoot / Root
A. With phosphate, *p*H 6·2:						
No silicate	86	9+5	12·02	3·03	15·05	3·97
Si≡$\frac{1}{5}P$	93	11+5	12·62	3·14	15·76	4·03
Si≡1*P*	91	10+4	12·69	2·59	15·28	4·90
B. With phosphate, *p*H 3·7:						
No silicate	64	11+3	9·54	2·27	11·81	4·21
Si≡$\frac{1}{5}P$	81	12+4	10·18	2·47	12·65	4·13
Si≡1*P*	81	11+3	9·96	2·22	12·18	4·49
A. Without phosphate, *p*H 6·2:						
No silicate	37	0+1	0·302	0·155	0·457	1·95
Si≡$\frac{1}{5}P$	54	1+0	0·649	0·239	0·888	2·72
Si≡1*P*	53	1+2	1·025	0·401	1·426	2·55
B. Without phosphate, *p*H 3·7:						
No silicate	27†	0+1	0·147	0·085	0·232	1·73
Si≡$\frac{1}{5}P$	27†	0+1	0·190	0·101	0·291	1·88
Si≡1*P*	33†	0+1	0·257	0·144	0·401	1·78

* First figure indicates shoots running up to ear; second figure small non-earing tillers.

† Approximately.

Statistical analysis of total dry weight data.

	With phosphate	Without phosphate: *p*H 6·2	Without phosphate: *p*H 3·7
Degrees of freedom	40	12	12
Standard deviation of mean = σm	0·6001	0·05652	0·02803
Standard deviation of difference between means = $\sigma(m_1 - m_2)$	0·8467	0·07993	0·03964
Difference required for a probability of ·05	1·7100	0·17425	0·08642
,, ,, ·10	1·3920	0·1425	0·0705

average of two tillers appeared. In this solution the addition of silicate caused distinct increase in the average height, green and dry weight of shoot and root, and in the ratio of shoot to root. The extra size of the root in comparison with the shoot was very noticeable in all cases in which phosphorus was absent. The total dry weight was doubled by the lower amount of silicate and tripled by the heavier dose, but even so the plants were very small and in no respect like those receiving phosphorus.

With *p*H 3·7 the beneficial effect of silicate was much less marked, the lower amount having little or no effect, though the height and

weight were increased somewhat by the heavier amount. In no case did the single shoot show any indication of proceeding to develop an ear.

In this case, therefore, silicate in the presence of phosphate, did little or nothing towards improving growth. In the absence of phosphorus considerable increase in dry weight was affected by the silicate in a solution of a favourable *p*H value, though less advantage was manifested in a more acid solution. These results suggest that under favourable conditions of absorption the silicon had been able to replace the missing phosphorus to some slight extent, and later work was directed towards the elucidation of this point.

(*c*) *Statistical notes.*

The statistical significance of the observed differences in mean dry weight may be estimated from the variation between replicates as follows. From each group of data within which comparisons of yield are to be made, a single estimate of the standard deviation of parallels is obtained by pooling the variances of all the cultures in the group. Thus there were in the whole experiment 10 cultures (each of 5 plants) receiving full phosphate supply: the standard deviation of parallels for the full phosphate group may thus be based on $40 = (10 \times (5 - 1))$ degrees of freedom. The standard deviation of the mean of 5 plants, σm, and of the difference between means of 5 plants, $\sigma (m_1 - m_2)$, are calculated from this in the usual way. If the standard deviation had been based on a very large population, a difference between means exceeding twice the standard deviation of the difference between means would occur by chance not more than once in twenty trials (*i.e.* Probability $(P) = \cdot 05$) and would be judged significant. When the number of degrees of freedom available for the estimate of the standard deviation is smaller we require more than twice the standard deviation to reach the same level of significance. The values of the factor, t, by which the standard deviation must be multiplied in order to obtain any given level of significance have been tabulated for small numbers of degrees of freedom by R. A. Fisher(4), and his tables have been used for these calculations. Values of $\sigma (m_1 - m_2)\, t$ for $P = \cdot 05$, *i.e.* values of a difference between means which will not be exceeded by chance more than once in twenty trials, are given, along with values of σm and of $\sigma (m_1 - m_2)$ at the bottom of Table I.

In the case of the culture solutions without phosphate the variation between replicates is much greater with the solution of *p*H 6·2 and the standard deviation has been estimated separately for each *p*H group.

The method outlined above for indicating the significance of the yield results will be used for all the experiments to be described in this paper and the values of $\sigma (m_1 - m_2) t$ for a probability of ·05 will be given for each group within which comparisons are to be made.

From the figures at the foot of Table I it is clear that the slight increase in mean dry weight due to the addition of silicate to culture solutions having full phosphate supply is without significance but that the increase due to silicate in the absence of phosphate is fully significant. Moreover the intermediate position occupied by the small dose of silicate (Si $\equiv \frac{1}{5}P$) leaves no doubt as to the association of increased increments of silicate with increased increment of yield.

With regard to the effect of the plants upon the *p*H value of the solution it was found that in the presence of phosphate the *p*H was rapidly changed to a uniform value of about 6·6, whether the original value was 3·7 or 6·2. In the absence of phosphorus the *p*H reached the same level of 6·6 when it had originally been 6·2, but striking variations occurred with the more acid solution. In this case where no silicate was added the *p*H changed from 5·8 to 6·6 with different plants during the first month, but afterwards the value changed much less and finally remained unaltered. With a light dressing of silicate the initial change was less marked, the value ranging from 4·8 to 5·9, after which it gradually approached the original 3·7, whereas with the heavier silicate the initial change was to 6·2, but less alteration occurred later, the final readings being from 4·8 to 5·6. Normally growing plants tend to stabilise the *p*H value of nutritive solution somewhat on the acid side of neutral, but in the absence of phosphate the normal functioning of the roots did not continue for long, and they became gradually less able to alter the *p*H, though with the heavier silicate this change was less marked.

II. *Experiment* 2, 1925.

In this second test, attention was concentrated on work with a single solution with and without phosphorus, the *p*H value being modified after the addition of silicate by HCl or NaOH to bring it to approximately 6·2. A certain latitude was allowed owing to the extreme difficulty of making exact adjustment at every change, but the general range was from *p*H 5·9 to 6·3, with occasional slight digression above or below. The amounts of silicate added were Si $\equiv 1P$, $\equiv 2P$, $\equiv 4P$ and in some cases $\equiv 8P$, $\equiv 16P$, with controls without silicate. The sodium silicate used in this case was the "C soluble silicate" (Brunner Mond) which was found to give the best results in soil cultures in 1924 (see

pp. 65, 66). In addition, a series was tested in which a very little phosphorus equal to the amount in ten barley grains (·00641 gm. P_2O_5) was added to each bottle. This represented ·0401 of the usual quantity of phosphate used in the nutrient solution, *i.e.* 159·9 mg. P_2O_5 per bottle or 266·5 mg. per litre.

Barley var. Spratt Archer. Graded: ·04–·05 gm. Sown: March 7th, 1925. Put into solution: March 17th, 1925. Harvested, 1st half: June 11th; 2nd half: July 28th, 1925.

Bottles of 600 c.c. capacity were used, and the solutions were the same as the A solutions (sodium chloride being omitted), with and without phosphate, in the 1924 experiment, with the addition of a third containing 6·41 mg. P_2O_5 per bottle. The plants taken off at the first harvest had six changes of solution, and those at the second harvest thirteen changes.

At intervals during the course of the experiment quantitative observations were made on all the plants with a view to obtaining an analysis of the effect of phosphate and of silicate upon the yield in terms of their effect upon the growth processes of the plant. Measurements were made of total height (to the tip of the longest leaf on the main shoot), width of leaves, number of tillers, and, in the later stages of growth, height of the ear. Development curves were thus obtained for certain aspects of growth. A discussion of the results for growth in height will illustrate the kind of information obtained by means of this technique.

(*a*) *Growth in height and leaf development.*

Fig. 1 shows the total height of certain selected cultures at intervals up to the time of the first harvest. In order to avoid confusion of the curves only six of the cultures are represented, showing the growth at the three levels of phosphate supply (full, little, none) and the effect of sodium silicate at each level[1]. For the cultures without silicate the curves run together until about the 20th day when a sharp divergence begins. As compared with the full phosphate culture that having a little phosphate lags markedly in the middle stages of growth but shows a recovery later, during the period of "shooting." The lag in the no-phosphate culture is still more marked and the recovery both very slight and longer delayed. There is in fact an actual decrease in height in the middle period owing to the shrivelling of the tips of the leaves. The effect of the addition of silicate (Si $\equiv 8P$) at this low level of phos-

[1] The silicate dressing which produced the maximum effect in each case is chosen for representation.

phate nutrition is to delay the marked divergence from the full phosphate culture until nearly the 30th day, but thereafter, although the advantage in height is maintained, the approximation to the full phosphate curve fails. The period of stationary growth is however shortened and the recovery is earlier and more marked than in the absence of silicate.

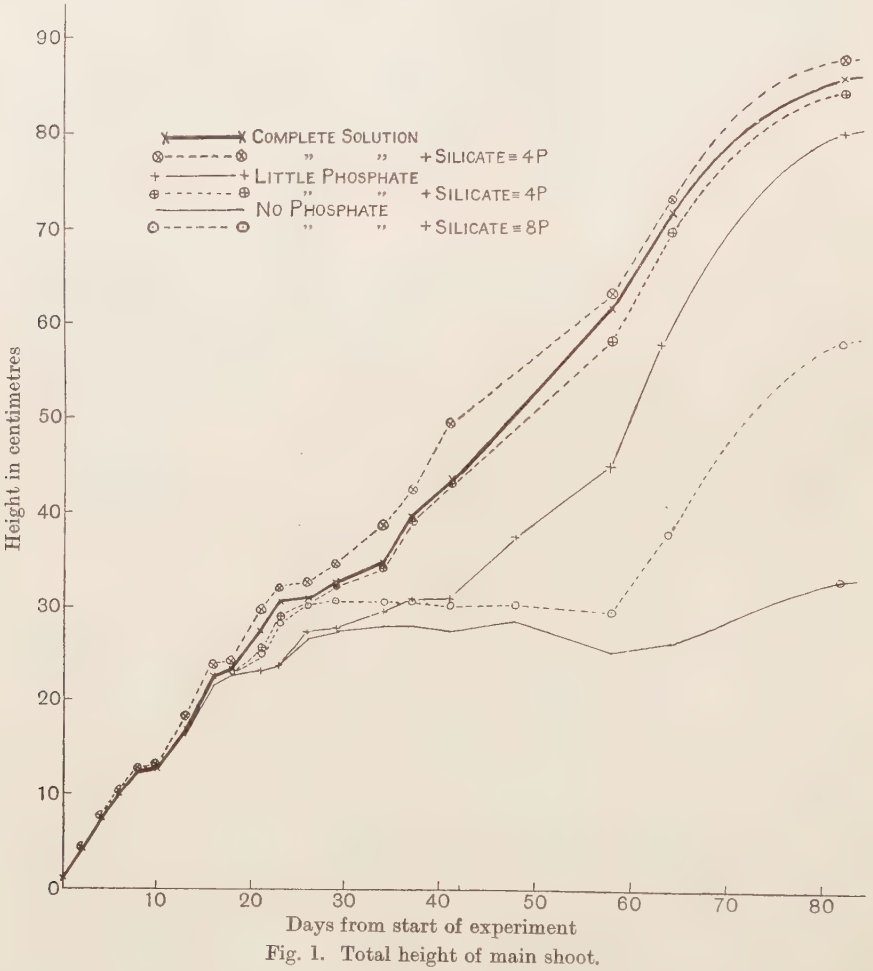

Fig. 1. Total height of main shoot.

The addition of silicate (Si $\equiv 4P$) to the culture having little phosphate, results in a very close approximation to the behaviour of the full phosphate culture, the lag having almost disappeared. The effect of the addition of silicate to the full phosphate culture is smaller but in

Table II.

Barley. Data at 1st harvest, 1925.

Average of five plants in each set.

Treatment	Dry weight gms. Shoot	Root	Total	Shoot / Root	Dry wt. Ears	% Ears / Total plant*	Significant difference between means $\sigma(m_1 - m_2)t$ for a probability of ·05: Total dry wt.	Ears as % of total dry wt.
With phosphate (159·9 mg. P_2O_5 per bottle). (Plate V, fig. 2):								
No silicate	10·32	1·39	11·71	7·42	0·521	4·06	2·952	2·402
Si≡ 1*P*	10·98	1·69	12·67	6·50	0·533	4·18		
Si≡ 2*P*	11·42	1·41	12·83	8·10	1·077	8·22		
Si≡ 4*P*	11·33	1·70	13·03	6·66	1·270	9·74		
No phosphate (Plate V, fig. 1):								
No silicate	0·257	0·092	0·349	2·79	0·003	0·89	0·1544	3·510
Si≡ 1*P*	0·308	0·093	0·401	3·31	0·014	3·45		
Si≡ 2*P*	0·389	0·121	0·510	3·21	0·026	4·71		
Si≡ 4*P*	0·557	0·152	0·709	3·66	0·056	7·63		
Si≡ 8*P*	0·723	0·242	0·965	2·99	0·121	12·69		
Si≡16*P*	0·612	0·172	0·784	3·56	0·055	6·45		
Little phosphate (6·41 mg. P_2O_5 per bottle):								
No silicate	2·16	0·32	2·48	6·75	0·196	8·87	0·876	4·425
Si≡ 1*P*	1·60	0·25	1·85	6·40	0·173	9·49		
Si≡ 4*P*	6·14	0·89	7·03	6·90	0·666	9·90		

* The figures in this column are the means of the values for the five plants in each set.

Table III.

Barley. Data at 2nd harvest, 1925.

Treatment	Dry weight gms. Shoot	Root	Total	Shoot / Root	Dry wt. Ears	% Ears / Total plant*	Significant difference between means $\sigma(m_1 - m_2)t$ for a probability of ·05: Total dry wt.	Ears as % of total dry wt.
With phosphate:								
No silicate	18·30	1·80	20·09	10·17	4·10	17·54	4·320	6·72
Si≡ 1*P*	24·27	2·36	26·63	10·28	6·29	23·63		
Si≡ 2*P*	21·93	2·37	24·30	9·25	6·13	24·63		
Si≡ 4*P*	20·84	1·92	22·76	10·85	6·49	28·54		
No phosphate:								
No silicate	0·284	0·078	0·362	3·64	0·022	5·50	0·1369	7·18
Si≡ 1*P*	0·439	0·100	0·539	4·39	0·060	10·92		
Si≡ 2*P*	0·558	0·150	0·708	3·72	0·088	12·48		
Si≡ 4*P*	0·699	0·197	0·896	3·55	0·153	16·98		
Si≡ 8*P*	0·871	0·162	1·033	5·38	0·294	28·30		
Si≡16*P*	0·586	0·100	0·686	5·86	0·105	13·64		

* The figures in this column are the means of the values for the five plants in each set.

the same direction, the improvement in growth being greatest in the middle period.

For the first 40 days of growth height measurements were taken at intervals sufficiently close to make possible a calculation of the change in rates of growth with time.

Fig. 2 shows the linear rate of growth in height (centimetres per day) for the two cultures with full phosphate (Si $\equiv$ 0 and Si $\equiv$ 4*P*) and the two with no phosphate (Si $\equiv$ 0 and Si $\equiv$ 8*P*). The two with "little

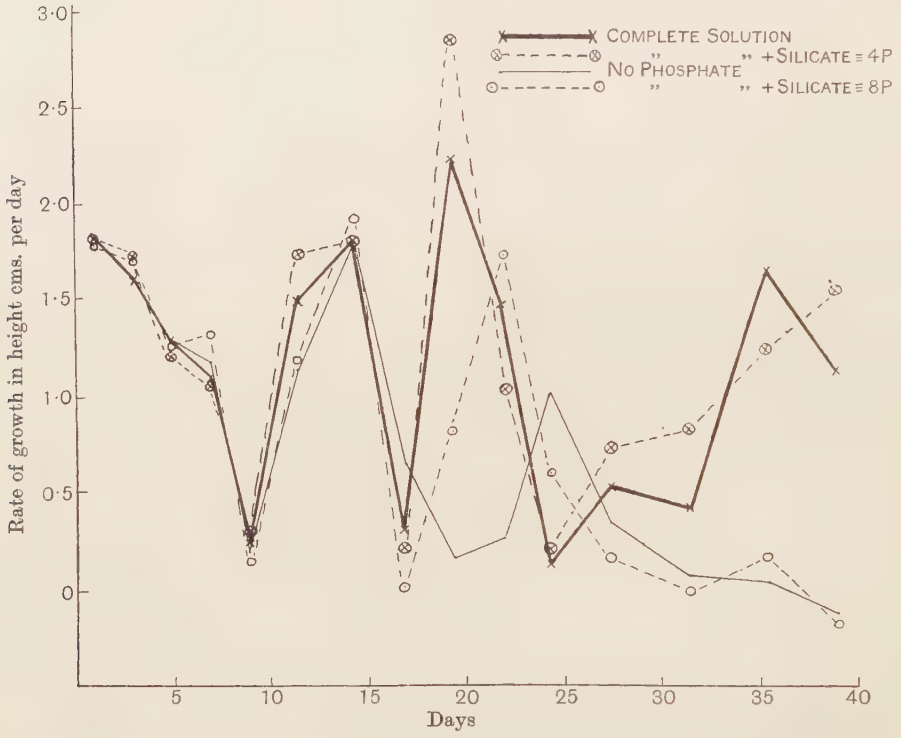

Fig. 2. Rate of growth in height as affected by phosphate and by silicate.

phosphate" are omitted to avoid confusion. Considering first the curve for full phosphate without silicate, the first part of the curve represents the close of the grand period of growth of the first leaf. The second peak of the curve belongs to the second leaf, which now overtakes the first leaf and becomes the longest; the third peak is similarly associated with the elongation of the third leaf. Owing to the facts that after this point younger leaves tend to overlap their predecessors while these are still growing appreciably and that individual plants in the set do not "keep step" quite so well, the grand periods of subsequent leaves become

merged, but the two slight peaks at 27·5 days and at 35·5 days correspond roughly to the growth of the 4th and 5th leaves. The curve for the no-phosphate culture, without silicate, shows, as compared with the normal, a progressive shift to the right in its peaks and a progressive diminution in general level. The effect of sodium silicate at this level of phosphate nutrition is to bring the curve back towards the normal. At the level of full phosphate supply the effect is again a shift to the left, though the difference is very slight.

It is quite clear that, beginning with the second leaf, the effect of phosphate deficiency is to delay the development of the leaves, while the addition of sodium silicate hastens their development. The following table, derived from periodic measurements of the lengths of the last three leaves on the main shoot, shows the time at which successive leaves as they emerged reached the same height as the leaf immediately preceding them and demonstrates this effect of phosphate and of silicate on the rate of emergence of leaves.

Table IV.

Rate of leaf development.

Days required for successive leaves to reach same height as leaf immediately preceding them.

	$L_2=L_1$	$L_3=L_2$	$L_4=L_3$	$L_5=L_4$	$L_6=L_5$
Full phosphate	10·0	19·5	28·5	33·75	40·0
„ $+Si\equiv 4P$	9·75	19·25	26·75	32·50	37·75
Little phosphate	12·0	23·0	36·0	43·0	55·0
„ $+Si\equiv 4P$	11·5	20·5	28·5	35·0	43·0
No phosphate	10·75	23·5	39·0	58·0	67·0
„ $+Si\equiv 8P$	10·75	20·5	35·5	48·0	58·25

It will be seen that for the stage when the 6th leaf has just become the longest leaf the lag in development for "little phosphate" is 15 days, and for no phosphate 27 days, these periods being reduced by the addition of silicate to 3 days and 18·25 days respectively.

To simplify discussion, data have been given for one concentration of silicate only at each level of phosphate supply: cultures with intermediate concentrations of silicate occupied intermediate positions for each of the aspects of growth considered, with the exception of the no-phosphate culture with 16 units of silicate, which was less advanced than the culture with 8 units and approximated to the culture with 4 units. (The yield of this cultnre shows a similar behaviour, see Tables II and III.)

The total number of leaves developed on the main shoot averaged ten and no consistent difference in total number as between plants with

and without phosphate could be established. The marked effect of silicate and of phosphate upon the time at which successive leaves emerge is thus accompanied by a similar effect upon the development of the final meristem of the main shoot, that is the ear. Developmental stages tend to be somewhat telescoped in the later stages of growth of the more backward plants, so that the lag in ear development is not as great (measured in days) as the lag in development of the 6th leaf. It is however very well marked and of considerable importance. At any one time a rough measure of the developmental stage reached by different plants may be obtained from the height of the "shoot" (combined leaf sheaths) relative to the total height, or of the height of the ear swelling within the "shoot" relative to the height of the "shoot." Data for the relative height of the ear in the main shoot for all cultures are given in Table V for a date just before the first harvest.

Table V.

Height of ear relative to total height, June 7th, 82 days from start.

Full phosphate	Si≡ 0	77·5 %	Height of ear is measured to middle of ear swelling.
	Si≡ 1	85·4	
	Si≡ 2	87·5	
	Si≡ 4	91·6	
Little phosphate	Si≡ 0	75·5	
	Si≡ 1	73·7	
	Si≡ 4	86·0	
No phosphate	Si≡ 0	70·0	
	Si≡ 1	70·5	
	Si≡ 2	71·5	
	Si≡ 4	73·0	
	Si≡ 8	77·9	
	Si≡16	71·7	

Subsequent measurements of the rate of emergence of the ears from the leaf sheath placed the cultures in exactly the same order. In the case of cultures without phosphate and with only small amounts of silicate the ears did not emerge above the last leaf sheath but protruded laterally through the slit in the sheath.

So far attention has been directed to the time relations of growth, and in particular of leaf development. The size of individual leaves is also affected by silicate and phosphate so that these substances increase the leaf area of the plant both by increasing the rate at which successive leaves emerge and by increasing the area of individual leaves. The following data for the width of the topmost fully unrolled leaf on May 14th, after 58 days' growth will illustrate this effect.

Leaf width.

	Units					
Si≡	0 cm.	1 cm.	2 cm.	4 cm.	8 cm.	16 cm.
Full phosphate	1·22	1·35	1·36	1·41	—	—
Little phosphate	0·89	0·86	—	1·16	—	—
No phosphate	0·568	0·640	0·608	0·720	0·720	0·770

(*b*) *Tillering.*

In view of the marked effect of phosphate and of silicate on the activity of leaf and ear meristems in the main shoot it is not surprising to find the initiation of tillers similarly affected. While, however, in the case of leaves and ears of the main shoot, the total number formed was not affected, in the case of tillering the number of meristems developed is markedly affected and results in considerable differences in total size of plants.

Table VI gives the number of side tillers per plant, for the six cultures previously considered, at intervals up to the time of the first harvest.

Table VI.

Tillers per plant (*average of* 5 *plants*).

	Days from start												1st harvest. Side tillers with ears
	21	23	26	29	34	37	41	48	58	64	82	86	
Full phosphate:													
Si≡0	0·2	0·7	1·3	2·2	3·3	4·1	5·2	6·2	8·0	9·0	8·9	8·8	7·4
Si≡4	0·8	1·0	1·9	2·3	4·3	5·5	7·2	8·7	11·0	10·5	8·8	8·4	7·6
Little phosphate:													
Si≡0	0·0	0·0	0·0	0·0	0·2	0·2	0·3	0·8	1·8	1·2	1·2	1·25	0·5
Si≡4	0·0	0·6	1·3	1·8	3·2	3·6	4·0	4·5	5·0	5·0	4·4	4·0	3·6
No phosphate:													
Si≡0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Si≡8	0·0	0·0	0·0	0·2	0·2	0·3	0·3	0·2	0·1	0·0	0·0	0·0	0·0

The similarity of the effect of phosphate and of silicate upon rate of tillering and number of tillers developed is evident from the table. What is even more marked is the close association between the level of phosphate supply and the effect of the silicate. Some tillering is indeed induced by the addition of silicate to cultures without phosphate but the tillers die and the effect is very slight. In the presence of little phosphate however, not only does tillering begin eleven days earlier but many more tillers are formed and most of them produce ears. With full phosphate tillering again begins earlier in the presence of silicate and the total number formed is increased. By the time of the first

harvest, the number is reduced, by the death of some, to the same level for both cultures. This number represents in the case of the silicate culture older and more advanced tillers. The average number of side tillers which produced ears was 7·5, a tiller number reached in the case of the silicate culture at 42 days, but reached in the case of the culture without silicate at about 54 days. The ears in the side tillers are in consequence further developed in the silicate cultures, and therefore, although at the first harvest the total number of ears is about the same for both cultures, yet the dry weight of the ears relative to the dry weight of the plant is more than doubled for the silicate culture (see Table II, p. 55). By the time of the second harvest most of the ears have reached maturity and the relative difference in ear weight between cultures with and without silicate is smaller though it remains in the same direction as before (Table II, p. 55).

The significance of the differences in total dry weight between different cultures will be discussed subsequently. It will be convenient however to conclude the discussion of the effect of silicate and of phosphate upon growth by considering the yield of grain.

Table VII.

2nd harvest.

		Dry weight of grain gm.	Grains per ear	Fertile %	Mean dry weight of single grain mg.
Full phosphate	(no Si)	2·358	7·2	28·8	32·75
„	Si≡ 1*P*	3·779	10·14	38·3	33·22
„	Si≡ 2*P*	3·917	9·70	37·3	35·40
„	Si≡ 4*P*	4·167	12·82	47·1	33·17
No phosphate	(no Si)	0·0	0·0	—	—
„	Si≡ 1*P*	0·0	0·0	—	—
„	Si≡ 2*P*	0·0062	0·2	—	31·00
„	Si≡ 4*P*	0·0477	1·4	—	34·07
„	Si≡ 8*P*	0·1882	10·8	—	15·00
„	Si≡16*P*	0·0288	3·6	—	7·70

Grain weight: significant difference between means for Full phosphate = 1·068; for No phosphate = 0·1732.

The improvement effected by silicate in rate of ear development is here apparent finally in yield per ear, an effect due mainly to an increase in the number of fertile grains, the mean grain weight being, except in the case of the no-phosphate cultures, relatively unaffected.

The emergence of the ears was adversely affected by the absence of phosphorus, as they tended to emerge sideways from the sheath, remaining partially enclosed at the upper end until cut. Gradual improve-

ment occurred in this respect with increasing amounts of silicate, till with Si ≡ 8P normal emergence was attained. Another marked tendency throughout was for the grain to ripen off without filling out properly, this again being improved by silicate.

So far, therefore, as concerns behaviour during growth the effects of phosphate and silicate are strikingly similar in type. The effect of the silicate is however quite definitely a function of the level of phosphate nutrition and, especially as concerns tillering, is relatively very small in the absence of phosphate from the solution.

(*c*) *Yield.*

The mean dry weights of the plants are given in Tables II and III, and the statistical significance of the differences in total dry weight may be judged from the last two columns which give the value of the difference between means which would not be exceeded by chance more than once in twenty trials. The effect of the silicate at each level of phosphate nutrition is as follows:

Complete phosphate. *1st harvest.*

There is no significant increase in mean dry weight due to silicate either for individual sets or for the average of all the sets with silicate, but the well-marked increase, with increasing silicate, of ear weight as a percentage of the total weight is quite significant.

2nd harvest.

Here the culture with Si ≡ 1*P* is significantly superior in dry weight to that without silicate but it cannot be distinguished from the other silicate cultures. The average of the silicate cultures is significantly greater than the no-silicate culture. Yield of grain moreover shows a significant increase with increasing silicate (see Table VII).

Little phosphate.

The yield for Si ≡ 1*P* is undoubtedly anomalous in being lower than for Si ≡ 0*P*, but the two yields do not differ by a significant amount and no importance can be attached to the apparent anomaly. The striking increase with Si ≡ 4*P* is quite significant.

No phosphate.

For both harvests. In the series of increasing silicate concentrations the yields increase up to Si ≡ 8*P* and then fall, and with the exception of Si ≡ 1*P* in the 1st harvest each step in the silicate series brings about a significant difference in yield. The depression in yield with Si ≡ 16*P* is quite significant.

It will be seen that the actual increments in yield due to addition of the same amount of silicate, *e.g.* Si ≡ 4*P*, are very small in the absence of phosphate (+ ·360 and + ·534), much greater in the presence of a little phosphate (+ 4·551) and still appreciable though less definitely significant in the presence of full phosphate (+ 1·32 and + 2·67). There is obviously no question of the *replacement* of phosphorus by silicon, but clearly the use that can be made of the phosphorus presented to the plant is affected by the addition of silicate.

(*d*) *Uptake of* P_2O_5, *of* SiO_2 *and of ash.*

The P_2O_5 and ash contents of the water culture plants from the first harvest are given in Table VIII. On account of the small weight of material available from the no-phosphate series the sets were bulked in pairs so as to have 3 samples in the series of increasing silicate concentrations.

Ash content. The ash content increases in every case with increasing silicate concentration, the increase being most marked in the roots. The large increase here may be partly due to silica adhering to the roots though great care was taken in washing them. The increase in ash content does not however contribute very greatly to the increase in dry weight of plants with addition of silicate as is shown by the figures for the ash-free dry weight.

Silica. The silica content of the roots was not determined owing to the doubt concerning silicate adhering to the root surface. In the shoots the SiO_2 content increases more than 10 times with the addition of Si ≡ 4*P*, but again contributes very little to the increase in dry weight.

P_2O_5 content. It will be seen that in spite of the fact that A.R. chemicals were used for the culture solutions, yet in the no-phosphate set the plants contain more P_2O_5 than was contained in the grain. The amounts concerned are very small and very slight impurity in the A.R. chemicals would account for the results. A test for phosphorus in the sodium silicate revealed no trace of that element and moreover evidence from the P_2O_5 content of the plants suggests that the effect of the addition of silicate upon the plants cannot be due to traces of P_2O_5 in the silicate. For, in the case of the little phosphate culture the addition of Si ≡ 4*P* results in an increased P_2O_5 uptake of 8·865 mg. while with the no-phosphate culture the increased uptake of P_2O_5 for Si ≡ 3*P* (Si ≡ 2*P* and ≡ 4*P* bulked) over Si ≡ $\frac{1}{2}P$ (Si ≡ 0*P* and ≡ 1*P* bulked) is only 0·239 mg. and that for Si ≡ 12*P* (Si ≡ 8*P* and ≡ 16*P* bulked) is only 1·078 mg. If the P_2O_5 were being supplied with the silicate the increase

in P_2O_5 uptake with addition of silicate should be greater with the cultures originally containing the smaller amount of phosphate.

With the no-phosphate solution therefore one is not working with zero concentration of phosphate, but with a very low concentration approaching zero[1]. In the solutions deficient in phosphate the effect of the addition of silicate is a progressive increase in uptake of P_2O_5. With the full phosphate solution however, the P_2O_5 content is decreased by the addition of silicate. The percentage of P_2O_5 both in dry weight and in ash content decreases with the addition of silicate. Calculated on ash free dry weight this decrease is smaller but still evident, *i.e.* the increase in ash free dry weight per plant due to the addition of silicate is greater than the increase in P_2O_5 per plant and from the point of view of dry weight production the P_2O_5 in the plant must be regarded as increasing in efficiency as silicate is added. The same feature, an increase in ash free dry weight greater than the increase in P_2O_5 content, is however found in the series of increasing P_2O_5 supply, the plants in the full phosphate solution having the lowest P_2O_5 percentage. In this respect the addition of silicate behaves like an increase in phosphate supply and the effect might be regarded as due to an increased availability to the plant of the phosphate already present in the solution. That there is an effect of this kind seems probable from other work, but the case of the full phosphate solution where, although the dry weight of ears is markedly increased by silicate yet the P_2O_5 content is reduced, suggests that the presence of silica does increase also the efficiency of the P_2O_5 within the plant.

Only a study of the P_2O_5 distribution in the plant and the changes in this distribution with time can clear up this question, but it may be suggested that the silica might act within the plant by unlocking phosphate from relatively quiescent parts of the plant and enabling it to be transferred to regions where assimilation and growth are active.

[1] An approximate estimate of the P_2O_5 concentration in the no-phosphate solution may be obtained from the three points on the curve relating yield to P_2O_5 supplied (otherwise than as impurity). The P_2O_5 supplied in mg. per bottle is 0, 6·41, 159·9, the yields are ·349, 2·48, 11·41. Solving the following equation for yield

$$\text{yield} = \frac{1}{k_1 + \frac{k_2}{P + p}},$$

where P is mg. P_2O_5 supplied and p is mg. P_2O_5 present as impurity (and in the seed) we have $k_1 = 0{\cdot}0703$, $k_2 = 2{\cdot}4215$, $p = 0{\cdot}8664$. The total weight of nutrient salts per bottle in the no-phosphate solution was 1·464 gm. so that an impurity of less than ·059 per cent. $\left(= \frac{{\cdot}8664 \times 10^2}{1{\cdot}464 \times 10^3}\right)$ would account for the results.

Table VIII.

Water cultures, 1924 *and* 1925.

P_2O_5 content, Ash content, and Silica content of plants.

	1925								1924		
	Full phosphate		Little phosphate			No phosphate			No phosphate		
	Si≡0	Si≡4*P*	Si≡0	Si≡1*P*	Si≡4*P*	Si≡0, 1*P*	Si≡2, 4*P*	Si≡8, 16*P*	Si≡0	Si≡$\frac{1}{5}$*P*	Si≡1*P*
P_2O_5 % of dry matter:											
Roots	0·657	0·482	0·484	0·456	0·388	0·386	0·404	0·378	—	—	—
Shoots	0·137	0·120	0·213	0·181	0·187	0·197	0·127	0·181	0·161	0·214	0·181
Whole plant	0·198	0·167	0·248	0·218	0·214	0·244	0·189	0·228	—	—	—
P_2O_5 content, mg. per plant:											
Roots	9·11	8·19	1·535	1·118	3·535	0·357	0·551	0·783	—	—	—
Shoots	14·16	13·60	4·600	2·905	11·460	0·556	0·601	1·208	0·735	1·900	2·580
Whole plant	23·27	21·79	6·135	4·023	14·995	0·913	1·152	1·991	—	—	—
Ash % of dry matter:											
Roots	19·10	36·20	6·740	6·360	16·780	10·20	18·80	30·00	—	—	—
Shoots	14·45	15·88	8·730	10·750	12·260	14·32	14·88	16·52	16·65	12·08	15·45
Whole plant	15·00	18·50	8·480	10·190	12·810	13·30	15·75	19·70	—	—	—
Ash free dry wt. gms.:										Shoots only	
Whole plant	9·972	10·632	2·265	1·670	6·120	0·3252	0·5138	0·7020	0·381	0·781	1·204
P_2O_5 % on ash free dry wt.	0·2335	0·2050	0·2708	0·2410	0·2450	0·2805	0·2242	0·2840	0·193	0·243	0·214
P_2O_5 % of ash wt. ...	1·323	0·902	2·93	2·14	1·662	1·830	1·200	1·158	0·966	1·72	1·170
SiO_2 % of dry matter:											
Shoots only	0·211	2·500	0·151	—	2·350	—	—	3·100	—	—	—
SiO_2 % of ash:											
Shoots only	1·46	15·75	1·73	—	19·20	—	—	18·75	—	—	—

Grain 1924. P_2O_5 % on fresh weight (approx.) ·762 %. Average weight of grain ·065 gm. P_2O_5 per grain = ·4953 mg.

Grain 1925. P_2O_5 % on fresh weight, 0·854 %. Average weight of grain ·045 gm. P_2O_5 per grain = ·3844 mg.

C. Fertilising Value of Sodium Silicate in Soil Cultures.

With a view to determining whether sodium silicate might be used instead of or in partial replacement of other artificial fertilisers, pot culture experiments were carried out in 1924 and 1925 with various combinations of silicates and manures. In 1924 two soluble silicates, C and M, and one insoluble glass silicate, M, were tested against controls without silicate for each manurial combination. The silicate was applied in quantities bearing the same proportion to the manures used as was the case in field experiments on the farm and provided silicon equivalent to five times the phosphorus in the 5 gm. superphosphate applied per pot. Nitrogen was supplied as sulphate of ammonia or dried blood, phosphorus as superphosphate or gafsa phosphate, and a test with dung was included. Rothamsted heavy loam was used, with the admixture of 10 per cent. sand to lighten it somewhat, and barley and mustard were grown at different seasons.

I. *Series I.*

(*a*) *Barley. Goldthorpe.*

Sown: Feb. 15th, 1924. Harvested: July 23rd, 1924.

The influence of silicate was not very marked during growth, though certain differences were shown by the dry weights.

Table IX.

Dry weights of barley.

Average of four pots of three plants in each set.

Manures	No silicate		C soluble silicate		M soluble silicate		M glass silicate		Significant difference in dry wt. (total shoot) $\sigma(m_1 - m_2)t$ for a probability of ·05
	Ears gm.	Total Shoot gm.	Ears gm.	Total Shoot gm.	Ears gm.	Total Shoot gm.	Ears gm.	Total Shoot gm.	
1. S/A, Super, K_2SO_4	30·53	60·48	35·80	**69·8**	35·2	**68·5**	31·8	62·0	5·325
2. Blood, Super and K_2SO_4	32·81	63·16	33·7	66·6	36·5	**70·4**	33·2	65·9	4·840
3. Super, K_2SO_4	20·74	*45·24*	24·8	**51·0**	21·9	48·2	21·2	48·0	4·170
4. S/A, K_2SO_4	30·11	59·81	32·5	**63·8**	31·9	**63·2**	31·5	**62·35**	2·203
5. S/A, Super	32·48	63·60	34·0	65·9	36·5	68·5	32·9	63·1	6·050
6. S/A, Gafsa and K_2SO_4	28·17	58·67	32·6	63·9	32·4	64·4	30·2	59·5	7·120
7. Dung	30·08	62·96	31·1	63·6	28·6	59·8	26·16	**56·62**	4·280
8. No manure	19·81	*43·31*	22·0	**48·9**	21·77	**48·02**	19·16	42·18	2·050
	Mean 57·15			61·685		61·371		57·465	1·610
							Average for whole experiment		4·550

The level of significance for each type of basal manuring is given in the last column of Table IX and in the columns for the three silicate manures, yields which differ significantly from the corresponding control are in heavy type. In the first column, for yield in absence of silicate, yields differing significantly from the yield for complete manure (No. 1) are in italics. The only basal manurings differing in yield from the complete manure are "no nitrogen" (No. 3) and "no manure" (No. 8), other variations in manuring being without effect. In the case of the "no manure" set the yield is increased by both the soluble silicates, the difference between no manure and complete being reduced by about 30 per cent. Much the same increase with silicate occurs in the no-nitrogen set, though here owing to the greater variation of parallel pots, only the increase due to the C soluble silicate is of undoubted significance.

For the other types of basal manuring we have a significant increase with all three silicates for the no-phosphate series (No. 4) and an increase with the soluble silicates for the no-potash series (No. 5): the latter increase is not however significant. The greatest increase is that given by the two soluble silicates in the presence of complete manure and is undoubtedly significant. On the whole the increases in yield are as marked with the more complete manures as with the deficient manures (with the exception of dung where the M glass silicate produces a significant depression). Averaging all the results the two soluble silicates produce a significant increase in yield of 7·8 per cent. while the M glass silicate is without effect. The proportion of dry matter in green was not affected by the soluble silicates but had a tendency to be higher with M glass silicate, especially in the absence of potash.

(*b*) *Mustard.*

Sown: Aug. 13th, 1924. Harvested: Nov. 13th, 1924.

Table X.

Average of four pots of three plants in each set.

	No silicate Dry wt. gm.	C soluble silicate Dry wt. gm.	M soluble silicate Dry wt. gm.	M glass silicate Dry wt. gm.	Significant difference in dry wt. $\sigma(m_1 - m_2)\,t$ for a probability of ·05
1. S/A, Super, K_2SO_4	23·98	22·48	24·28	22·24	2·99
2. Blood, Super, and K_2SO_4	*19·53*	**23·13**	20·08	20·58	2·20
3. Super, K_2SO_4	*5·43*	5·49	5·08	4·98	0·68
4. S/A, K_2SO_4	*14·88*	20·90	12·95	15·45	6·28
5. S/A, Super	*20·05*	23·15	22·58	22·03	3·42
6. S/A, Gafsa, and K_2SO_4	*20·32*	20·83	17·95	18·39	4·21
7. Dung	*6·03*	5·65	6·10	6·25	1·154
8. No manure	*5·05*	**7·28**	5·45	5·63	1·145
Mean	14·41	16·12	14·31	14·44	1·081

Average for whole experiment 3·065

As before, yields with silicate differing significantly from the yields of the corresponding control (Table X) are in heavy type, and yields without silicate differing significantly from the complete manure (No. 1) are in italics. Those under which there is a broken line show differences (from their control) for which P (Probability) lies between ·05 and ·10.

In marked contrast to the results with barley on the same soil all manurings other than the complete (No. 1) give significantly lower yields, and it is only upon these deficient manurings and not at all upon the complete manuring that silicate has any effect, and then only the C soluble form is effective. The manurings which show a response to this silicate are "no manure" (No. 8), where the increase is quite small but significant, "no superphosphate" (No. 4), where the increase is considerable and probably though not certainly significant, no potash (No. 5), where the increase is small and of doubtful significance, and dried blood (No. 2), where the increase is small but significant. So far as they go the results with mustard are consistent with the theory of a replacement of phosphate and possibly of potash manures by silicate.

Considering the results from both crops it is evident that the soluble silicates were the more active, especially the C soluble form, and that they tended to cause increase in dry weight with deficient mineral manuring, especially phosphorus, and occasionally benefit occurred even with complete manuring.

II. *Series II.*

As silicates had proved capable of partially replacing phosphorus, and possibly potash, in artificial fertilisers, an attempt was made in 1925 to determine whether such replacement could be made economically, and the proportion of silicate required for the purpose. In order to give the sodium silicate full play special Cheshire soil was obtained, deficient in phosphate, potash and lime. The lime deficiency was corrected by the addition of sufficient calcium carbonate to bring the *p*H value of the soil to about 7·0. The unit of silicate applied was that containing as much silicon as is equivalent to the phosphorus in 5 gm. 15·93 per cent. superphosphate. This unit was adopted for convenience, as the complete fertiliser applied to each pot containing 21 lbs. soil consisted of

5 gm....	...	...	...	15·93 per cent. Super.
2 gm....	...	...	...	K_2SO_4
2·5 gm.	...	...	...	S/A

The range of silicate tested was

$$\text{Si} \equiv 0P, \equiv 1P, \equiv 2P, \equiv 4P, \equiv 8P, \equiv 16P.$$

The actual quantities of the C soluble silicate applied per pot were therefore

$0P$, none; $\equiv 1P$, 1·324 gm.; $\equiv 2P$, 2·648 gm.; $\equiv 4P$, 5·296 gm.; $\equiv 8P$, 10·592 gm.; $\equiv 16P$, 21·184 gm.

The addition of so much silicate had a considerable effect on the *p*H value of the soil, and had this not been compensated for, it would have been impossible to estimate how far the results were influenced by varying alkalinity. Sufficient hydrochloric acid was therefore added to neutralise the silicate in each case. To ensure adequate distribution the silicate for each pot was mixed with a small quantity of dried soil, the acid (made up to a standard quantity throughout to avoid affecting the water content) was pipetted over and the whole thoroughly incorporated. This was then sprinkled over the bulk of the soil, with the requisite manure, and all well mixed together. In this way it is hoped that the formation of acid and alkaline "pockets" in the pots was avoided, and a uniform distribution of silicate and fertilisers obtained.

(*a*) *Barley. Spratt Archer.*

Seed graded: ·04–·05 gm. Seed sown: March 13th, 1925. Harvested: July 31st, 1925.

The manurial scheme was:

1. No manure.
2. 2·5 gm. S/A, 2 gm. K_2SO_4.
3. 2·5 gm. S/A, 2 gm. K_2SO_4, ·0241 gm. Super.
4. 2·5 gm. S/A — 5 gm. Super.
5. 2·5 gm. S/A, 2 gm. K_2SO_4, 5 gm. Super.

(*a*) *Growth and maturity.* During the growth the most noticeable feature was the general, though not universal, improvement with increasing doses of silicate. This was particularly striking, and somewhat unexpected, with the unmanured plants, which reached a development with heavy dressings of silicate approximating to that of plants receiving nitrogen and potash in addition. At the time of harvesting all plants receiving phosphate, with or without potash, were nearly dead ripe, irrespective of the silicate dressing. With N and K, but no P, maturity was much less advanced, but improved with increasing silicate, though even with Si $\equiv 16P$ much green colour was still observable in leaves and ears. With no manure at all, *i.e.* in absence of N and K as well as P, all plants were a little riper than those receiving N and K, and again ripeness increased with silicate.

Silicate, therefore, acted in the same sense as phosphate, in hastening maturity, although even with the heaviest dressing it was less effective in that respect than a single dose of 5 gm. superphosphate.

(β) *Discussion of dry weights.*

Table XI.

Barley. Total plant. Dry weight.

Average of four pots of three plants in each set.

	Si	≡0P gm.	≡1P gm.	≡2P gm.	≡4P gm.	≡8P gm.	≡16P gm.	Significant difference $\sigma(m_1 - m_2)t$ for a probability of ·05
1. No manure		17·341	22·699	21·179	24·653	29·570	30·609	5·55
2. N + K (no P)		30·695	30·249	32·932	29·719	31·437	32·574	3·07
3. N + K + little P		29·569	29·019	31·092	31·300	30·604	33·005	4·17
4. N + P (no K)		34·854	41·961	45·838	47·065	47·240	51·807	5·51
5. N + K + P		48·490	49·078	50·088	49·118	50·139	54·776	4·27
							Average for whole experiment	4·435

Table XII.

Barley. Ears. Dry weight.

	Si	≡0P gm.	≡1P gm.	≡2P gm.	≡4P gm.	≡8P gm.	≡16P gm.
1. No manure		7·459	10·716	9·342	11·462	14·552	14·103
2. N + K (no P)		12·366	12·271	13·478	12·750	13·741	14·303
3. N + K + little P		12·149	11·065	13·502	13·258	13·573	14·578
4. N + P (no K)		16·984	21·104	23·972	23·935	23·093	22·994
5. N + K + P		23·390	24·268	24·092	22·730	23·175	22·021

Table XIII.

Barley. Grain. Dry weight.

	Si	≡0P gm.	≡1P gm.	≡2P gm.	≡4P gm.	≡8P gm.	≡16P gm.	Significant difference $\sigma(m_1 - m_2)t$ for a probability of ·05
1. No manure		5·721	9·123	7·449	9·110	11·668	11·296	2·64
2. N + K (no P)		9·996	9·467	10·469	10·309	10·648	11·646	1·76
3. N + K + little P		9·583	8·462	10·959	10·064	10·874	11·667	1·89
4. N + P (no K)		13·555	17·093	20·151	19·275	18·744	18·252	3·315
5. N + K + P		18·904	20·001	19·474	18·780	19·000	17·731	3·095
							Average for whole experiment	2·498

With barley, on the light Cheshire soil, deficient in available phosphorus and potash, silicate failed to bring about any appreciable improvement when phosphorus was omitted from the manure, and it was therefore not replacing the phosphorus *per se* nor unlocking stores of

phosphorus in the soil and rendering them available as plant food (Table XI). The large increase with silicate when *potash* was omitted suggests that the sodium silicate was either replacing or unlocking potash (Plate V, fig. 3). The presence of the base sodium as well as silicon renders it impossible to determine which of the elements was the active agent, or whether the compound as such was effective. It is conceivable that the sodium might have functioned as potassium in the economy of the plant or that it might have replaced potassium in soil compounds, thus freeing the latter element and rendering it available for use. On the other hand, the silicon may have played some part in plant nutrition or soil conditions for which no explanation can be offered. The heavy increase with silicate in the absence of any other fertiliser is noteworthy, as the Si $\equiv 16P$ was as effective as a dressing of N and K, and nearly as much so as N + P, and it is difficult to formulate a reason for so marked a beneficial action. Unfortunately owing to limitations of space no tests were made with K and P in the absence of nitrogen, so no information is available as to the need of the soil for nitrogenous manuring.

In view of the marked effect, noted in the water culture experiments, of silicate upon ear formation and on the percentage of fertile florets a careful examination of all the ears of the plants in this experiment was made (Tables XII and XIII). It will be convenient to summarise the results in two tables showing (1) the average effect of silicate irrespective of other manuring (Table XIV), (2) the average effect of other manuring irrespective of silicate (Table XV).

Table XIV.

A. *Effect of silicate dressing.*

Si	$\equiv 0P$	$\equiv 1P$	$\equiv 2P$	$\equiv 4P$	$\equiv 8P$	$\equiv 16P$
Ears per pot	14·95	15·77	17·39	16·95	17·50	18·05
Florets per ear	29·46	29·66	27·50	28·46	28·88	29·12
Fertile florets (%)	82·24	81·82	81·74	77·86	77·08	72·12
Average grain wt., mg.	30·43	32·80	33·89	35·41	36·33	37·62

Table XV.

B. *Effect of basal manuring.*

	No manure	No P	Little P	No K	Complete
Ears per pot	11·93	14·24	13·97	21·78	21·92
Florets per ear	28·53	28·14	28·23	29·62	29·80
Fertile florets (%)	78·97	81·02	82·58	76·73	74·58
Average grain wt., mg.	33·16	31·78	31·60	36·40	39·15

The major effect of silicate dressing and of variation in basal manuring is on the number of ears per pot; upon the average number of florets per ear neither has much effect but both again affect the percentage of fertile florets, which decreases with increased silicate and with the addition of superphosphate and potash. This decrease runs parallel with the increase in the number of ears and reduces the grain yield below what would be expected from the increase in ear number. A further compensation, however, is found in the average weight of grain, which is affected by both silicate and the other manures in exactly the opposite direction to percentage fertility.

Taking the two basal manures which showed the greatest response to silicate dressings, no manure (Table XVI) and no potash, the major part of the increase in grain yield with increasing silicate can be ascribed to an increased number of ears per plant, while the effects upon percentage of fertile florets and upon average grain weight are smaller and tend to counterbalance one another.

Table XVI.

Effect of silicate in presence of no manure.

Si	≡0*P*	≡1*P*	≡2*P*	≡4*P*	≡8*P*	≡16*P*
No. of ears	9·25	11·3	11·7	12·25	12·75	14·25
Florets fertile (%)	82·5	76·0	86·0	86·3	74·7	80·6
Average grain wt., mg.	27·9	33·4	32·1	34·3	34·8	36·3
Grain yield, gm.	5·72	9·12	7·45	9·11	11·67	11·30

Effect of silicate in presence of no potash.

No. of ears	17·5	20·75	21·50	23·25	22·75	25·00
Florets fertile (%)	79·9	82·9	85·00	74·3	74·2	64·1
Average grain wt., mg.	31·4	33·9	36·9	37·7	38·1	40·4
Grain yield, gm.	13·55	17·09	20·15	19·28	18·74	18·25

Thus the results for pot cultures differ considerably from those for water cultures, where the main effect of silicate upon yield of grain lay in an increase in number of fertile grains and not at all in increased number of ears or increased grain weight. Comparison between the two sets of conditions is however difficult, and as regards percentage of fertile grain it is clear that the conditions were very different, since the percentage fertility ranges from 28 to 47 per cent. in the case of the water cultures and from 64 to 88 per cent. in the pot cultures. The very small effect of silicate in the absence of superphosphate, an effect moreover revealed also by the analysis of the grain yields, is also surprising.

(*b*) *Mustard. Brassica alba.*

Seed sown: Aug. 20th, 1925. Harvested: Nov. 24th, 1925.

This was a repetition of the last described barley experiment with the addition of sets containing two intermediate quantities of superphosphate, to determine the effect of silicate in the presence of various smaller amounts of phosphate than that normally applied.

(*α*) *Growth and development.* As with barley, the plants showed a very general improvement with increasing doses of silicate, except that in several cases a depression was noticed on the addition of Si $\equiv 1P$ and $\equiv 16P$. A curious point was the sudden improvement with Si $\equiv 1P$ when no manure was applied, the plants forging ahead of those receiving N and K with superphosphate up to 0·2 mg. per pot. This was not observed with any other dressing of silicate. Owing to the lateness of the season the flowering stage was not reached, but buds were formed in the sets receiving full dressing of P and N with and without K. After about two months' growth brownish patches appeared on the edges of all manured sets receiving no K, irrespective of the silicate dressing, and occasionally slight browning of the edges occurred with full manure or that providing 1 gm. super. The injury, once manifest, did not spread so as to involve more of the leaves, and was thus less noticeable when the plants were harvested than earlier in their development. Slight aphid attack developed once or twice, but was checked by spraying with soft soap and very little nicotine. The time of harvesting was decided by the fact that the lower leaves of most plants began to turn yellow and fall off, owing to the lateness of the season. All plants when cut were still green and healthy and actively growing.

(*β*) *Discussion of dry weights.*

Table XVII.

Mustard Total plant. Dry weight.

Average of four pots of three plants in each set.

Si	$\equiv 0P$ gm.	$\equiv 1P$ gm.	$\equiv 2P$ gm.	$\equiv 4P$ gm.	$\equiv 8P$ gm.	$\equiv 16P$ gm.	Significant difference $\sigma(m_1-m_2)\,t$ for a probability of ·05	Variance of parallels (means of 4 pots)
1. No manure	5·428	8·003	7·740	8·450	10·108	11·675	2·23	0·5631
2. N+K (no P)	6·523	5·448	8·475	9·150	12·218	15·408	3·68	1·5303
3. N+K+0·0241 gm. Super	7·023	5·625	9·363	11·810	13·845	11·740	4·35	2·1405
4. N+K+0·2 gm. Super	8·970	7·598	10·233	11·383	13·525	11·573	3·84	1·6725
5. N+K+1·0 gm. Super	14·593	12·760	16·433	18·465	23·253	20·475	5·04	2·8747
6. N+K+5·0 gm. Super	27·670	24·195	27·065	23·293	29·580	27·495	5·85	3·8715
7. N+P (no K)	22·243	24·658	26·538	25·243	28·813	25·553	5·16	3·0173
						Average for whole experiment	4·24	2·2421

It has already been shown that with barley the omission of either potash or phosphorus from a complete manure (without silicate) had a very similar effect in depressing the dry weight, the absence of P being a little more harmful. With mustard, on the same light Cheshire soil, the omission of phosphorus vitiated the beneficial action of the added N and K, so that the dry weight rose very little above that when no manure at all was added. With increase in the phosphate supplied a gradual improvement in dry weight set in. The addition of silicate brought about a greater increase of dry weight with deficient phosphorus than with deficient potash, suggesting that in this case the silicate is either releasing some of the phosphorus locked in the soil, or that the silicon is partially replacing phosphate (Plate V, fig. 4). With mustard, as with barley, the considerable increase with silicate in the absence of any other fertiliser is noteworthy, the addition of $\mathrm{Si} \equiv 16P$ being as effective as a dressing of N and K with some amount of superphosphate between 0·2 and 1·0 gm. per pot.

Comparison may be made of the effect of silicate on the heavy unlimed Rothamsted soil and the light limed Cheshire soil in which the alkalinity induced by the silicate was rectified by the addition of hydrochloric acid. With *barley* on both soils silicate was beneficial in differing degrees in the absence of manure and also with complete fertilisers. In the absence of potash, however, silicate was less beneficial on the Rothamsted soil than on the Cheshire type, whereas in the absence of phosphorus it did cause a slight improvement, the difference in the potash and phosphorus needs of the two soils being probably sufficient to explain this variation. With *mustard* the results were similar on both soils, except that as the Rothamsted soil was initially less deficient in phosphorus the extreme depression in its absence from a mixed manure was less evident.

(γ) *Statistical consideration of results.* The general similarity of the effect of silicate and of phosphate upon yield is clear from the figures in the table. The series of increasing doses of superphosphate was introduced in the hope of enabling one to characterise more precisely the type of interaction between silicate and phosphate that is involved. From the point of view of the yield-factor relationship[11] two simple alternatives present themselves: (1) the addition of unit amount of silicate is equivalent to the addition of a fixed amount of superphosphate, (2) the addition of unit amount of silicate is equivalent to increasing the amount of superphosphate already present in a fixed ratio. In the first case we might suppose that unit amount of silicate could replace so much

phosphorus within the plant or could set free from the soil so much phosphate. In the second case we might suppose that the efficiency of the phosphorus within the plant was increased in a fixed ratio by the presence of so much silicate or that the fraction of the total superphosphate supply which is available to the plant is similarly increased in a fixed ratio.

On the first supposition the silicate effect might be described in terms of "equivalent increments of superphosphate," on the second it would be described in terms of "relative efficiency of superphosphate." The difference between the two alternatives may be appreciated by

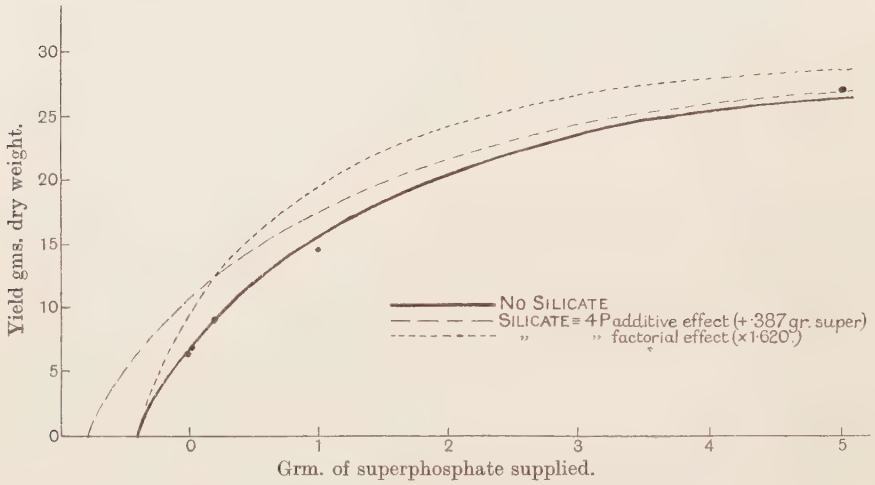

Fig. 3. Mustard. Yield—superphosphate relationship.

reference to Fig. 3 where the continuous line represents the simple yield-superphosphate relation for this experiment, and the dotted lines show how the uniform addition of a fixed dose of silicate would on either of the above suppositions affect the yield[1]. With the exact form of the yield-superphosphate relation we are not for the moment concerned, other than to note that it is of the "diminishing returns" type. The important point is that if the silicate effect is represented as equivalent to an increment of superphosphate then the yield curve is simply shifted to the left, with the consequence that the increment of yield due to silicate is maximal at zero superphosphate supply and rapidly diminishes as superphosphate increases. If it is, however, an effect on the

[1] The two curves for the effect of silicate are those for the effect of four times the unit dose of silicate in this experiment, and are based on Table XIX A and B.

efficiency of the superphosphate already present, then the origin of the yield curve remains fixed but the scale of the horizontal axis contracts, so that the increment of yield due to silicate is zero at zero superphosphate, increases at first with increasing superphosphate, and then diminishes again to zero.

We saw earlier (p. 54) that the water culture results with barley were of the second type, the increase in yield with silicate being quite small with "no phosphate," very considerable with little phosphate, and small with full phosphate. Again the evidence from the P_2O_5 content of the water culture plants suggested an effect of silicate upon the efficiency of phosphorus within the plant, which would be consistent with this type of yield relationship.

The yield data for the mustard may now be examined. The first essential is an equation for the yield-superphosphate relation. Several types of equation, including the well-known equation of Mitscherlich are available. There are reasons (11) however for preferring an equation of the form Y (Yield) $= \dfrac{1}{k_1 + \dfrac{k_2}{p + P}}$, where k_1 and k_2 are constants, P is the amount of superphosphate added to the soil, and p is the equivalent amount of superphosphate already present in the soil. The effect of the addition of silicate is to be represented either as an addition, P', to the function $(p + P)$, or as the multiplication of the function $(p + P)$ by a definite factor E. The procedure adopted has been to calculate the constants for the 5 yields for increasing superphosphate without silicate and to apply this equation to the yields with silicate. Selection of all possible groups of 3 observations from the 5 observed yields gave 10 sets of simultaneous equations from each of which by eliminating k_1 and k_2 a value for p was obtained. These 10 values were weighted by the mean yield for each of the 10 sets of observations and the weighted mean of these values taken to form a new set of equations linear in form from which Gauss' least square method k_1 and k_2 are found. The calculated equation is Yield $= \dfrac{1}{\cdot 02922 + \dfrac{\cdot 0503}{\cdot 406 + P}}$, the calculated yields being 6·52, 6·84, 8·90, 15·38, 25·99 and the observed yields 6·523, 7·023, 8·970, 14·590, 27·670. The sum of squares of deviations of calculated from observed values is 3·4845 and the variance is half this—1·74225—since after fitting 3 constants only 2 degrees of freedom are left. For the whole experiment the variance of parallels is 2·2421 (126 degrees of

freedom), *i.e.* greater than the variance of deviations from this fitted equation: the variance of parallels for the 5 sets without silicate involved in the fit is however much smaller 1·2657 (15 degrees of freedom). The divergence between the variance of parallels and that of deviations is not however significant.

The calculated equation may accordingly be used as a basis for the analysis of the silicate effect. For the moment the series of silicate dressings with full superphosphate manuring will be omitted from consideration since the variation due to silicate dressing within that set is very little greater than the random variation of parallels. For the other levels of superphosphate manuring the value of $P + P'$, *i.e.* the equivalent amount of superphosphate required to give the observed yield for each combination of silicate and superphosphate, may be calculated. (In the equation, k_1, k_2 and p being fixed, $P + P'$ determines Y and Y determines $P + P'$.)

Table XVIII.

Calculated values of $P + P'$.

Superphosphate (P) gm.	Units of silicate 1	2	4	8	16
0·0	−0·080	0·161	0·222	0·551	1·003
0·024	−0·068	0·242	0·501	0·765	0·493
0·20	+0·084	0·331	0·435	0·717	0·475
1·00	+0·617	1·188	1·615	3·220	2·158

Subtraction of the amount of superphosphate supplied (P) expresses the silicate effect as an "equivalent increment of superphosphate." Division of the calculated value $p + P + P'$ by the value of $p + P$ for no silicate expresses the silicate effect in terms of the "relative efficiency of the superphosphate."

Table XIX.

A. *Silicate effect as an equivalent increment in superphosphate.*

Superphosphate (P) gm.	Values of P' Units of silicate 1	2	4	8	16	Mean
0·0	−0·080	0·161	0·222	0·551	1·003	0·3714
0·024	−0·092	0·218	0·477	0·741	0·469	0·3626
0·20	−0·116	0·131	0·235	0·517	0·275	0·2084
1·00	−0·383	0·188	0·615	2·220	1·158	0·7596
Mean	−0·168	0·1745	0·387	1·007	0·726	

B. *Effect of silicate on relative efficiency of superphosphate.*

Values of E in terms of unity for Si≡0.

Superphosphate gm.	Units of silicate 1	2	4	8	16	Mean
0·0	0·803	1·394	1·545	2·355	3·470	1·9134
0·024	0·785	1·508	2·110	2·730	2·090	1·8446
0·200	0·808	1·215	1·387	1·855	1·455	1·3440
1·00	0·726	1·133	1·440	2·578	1·825	1·5404
Mean	0·7805	1·3125	1·6205	2·3795	2·2100	

As would be expected from the random variance of the yields from which these tables are calculated, in both cases the values for any one silicate dressing vary rather widely from one another. The total variation shown by each set of 20 values may be analysed as follows:

Table XX.

Analysis of variance.

A. *Table of "equivalent increments."*

	Sum of squares	Degrees of freedom	Variance
Total	6·05174	19	
Silicate dressing	3·35156	4	0·83789
Superphosphate	0·82830	3	0·27610
Differential	1·87188	12	0·15599
Superphosphate + differential	2·70018	15	0·18001

$$z' = \tfrac{1}{2}\log_e \frac{0{\cdot}83789}{0{\cdot}18001} = 0{\cdot}76908$$

B. *Table of "relative efficiencies."*

	Sum of squares	Degrees of freedom	Variance
Total	10·04750	19	
Silicate dressing	6·86404	4	1·71601
Superphosphate	1·06225	3	0·35075
Differential	2·12121	12	0·17677
Superphosphate + differential	3·18346	15	0·21223

$$z' = \tfrac{1}{2}\log_e \frac{1{\cdot}71601}{0{\cdot}21223} = 1{\cdot}0447$$

In both cases the greater part of the total variation is due to variation in silicate dressing while variation in superphosphate has a much smaller effect. The residue after accounting for the variation due to silicate is however relatively very much smaller for the second table as may be seen by comparing the values of z[1] for the two tables. This table, therefore, which represents the effect of silicate as equivalent to a definite change in the efficiency of the superphosphate present, gives the more adequate account of the data.

A further test of the two alternatives lies in the comparison of the observed yields with those calculated on the assumption that the effect

[1] z is the natural logarithm of the ratio between the two standard deviations and is used in estimating the significance of any difference between them (*v.* R. A. Fisher, *loc. cit.* p. 192.)

of silicate can be represented by the mean figures for "equivalent increment" or for "relative efficiency" given at the foot of Tables XIX, A and B: the two equations are

$$(1)\quad \text{Yield } (Y) = \frac{1}{\cdot 02922 + \dfrac{\cdot 0503}{\cdot 406 + p + P'}}.$$

where for Si = 0, 1, 2, 4, 8, 16 units, P' takes values

$$0, \; -\cdot 168, \; \cdot 1745, \; \cdot 387, \; 1\cdot 007, \; \cdot 726.$$

$$(2)\quad \text{Yield } (Y) = \frac{1}{\cdot 02922 + \dfrac{\cdot 0503}{(\cdot 406 + P) \times E}},$$

where for Si = 0, 1, 2, 4, 8, 16 units, E takes values

$$1, \; \cdot 7805, \; 1\cdot 3125, \; 1\cdot 6205, \; 2\cdot 3795, \; 2\cdot 2100.$$

The calculated yields are given in the following table.

Table XXI.

Calculated yields: (*a*) *equation* (1), (*b*) *equation* (2).

	Silicate					
	0		1		2	
Superphosphate	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)
gm.						
0·0	6·52		4·15	5·31	8·60	8·08
0·024	6·84		4·52	5·57	8·89	8·44
0·200	8·90		6·92	7·38	10·68	10·81
1·000	15·38		14·31	13·30	16·40	17·69
5·000	25·99		25·72	24·25	26·18	27·55
	4		8		16	
	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)
0·0	10·80	9·46	15·42	12·30	13·60	11·72
0·024	11·02	9·84	15·60	12·72	13·77	12·16
0·200	12·52	12·41	16·58	15·60	14·96	14·96
1·000	17·32	19·50	19·98	22·58	18·94	22·00
5·000	26·40	28·60	27·00	30·18	26·70	29·88

For each equation 5 constants additional to the original 3 have been calculated from the data, so that for the estimation of the variance of deviations from the expected yields we have, for the set of treatments silicate 0–16 units and superphosphate 0–1·0 gm., 24–8 = 16 degrees of freedom. The variance of deviations (sum of squares divided by the number of degrees of freedom) for the first equation is 3·8754, for the

second it is 2·7533. The random variance of parallels for this group of pots is 2·0607 (72 degrees of freedom) so that for the first case

$$z = \tfrac{1}{2} \log_e 3{\cdot}8754/2{\cdot}0607 = {\cdot}3156,$$

for the second $z = \tfrac{1}{2} \log_e 2{\cdot}7533/2{\cdot}0607 = {\cdot}1448.$

The value of z required for a probability of ·05 is about ·289[1], so that the deviation between observed and calculated yields in the first case is greater than would be expected by chance once in twenty trials and must be considered significant. The deviations from the second equation are greater than the random deviations, but not significantly so, and this equation may be accepted as an approximate representation of the observed facts.

So far we have dealt only with the yields which have been used in calculating the constants of the equation. We may calculate also yield values for complete superphosphate and silicate. (These are given in the lowest row of Table XXI.) The variance of deviations (for the second equation) now becomes 3·6805 (22 degrees of freedom) while that of parallels is 2·4228 (90 degrees of freedom). For these variances $z = 0{\cdot}209$ while for a probability of ·05 the value of z required is 0·2537 so that the deviations are still not significant.

Of the whole experiment we are now left with the no-potash and the no-manure series. If these show the same silicate-phosphate interaction as the other series the same equation should serve with an alteration only in the constant k_1 which is not associated with the silicate-superphosphate function.

For the no-potash set calculation gives $k_1 = {\cdot}031404$ instead of ·02922 and for the calculated yields we have (for Si = 0, 1, 2, 4, 8, 16), 24·043, 22·661, 25·400, 25·912, 27·205, 27·068. The variance of deviations of this series 2·7182 (with 5 degrees of freedom) is less than the variance of parallels which is 3·0173. For the whole experiment, excluding the no-manure series, we have the variance of deviations 3·5023 and the variance of parallels 2·5219 (108 degrees of freedom). For these variances $z = {\cdot}1647$, whereas for a probability of ·05 z requires to be $= {\cdot}2314$. Thus the slight observed effect of silicate upon yield in the absence of potash, can be represented as the effect of the silicate upon the efficiency of the superphosphate supplied and no sodium-potassium interaction is suggested.

The value of k_1 for the no-manure series is calculated as ·03837 and the calculated yields (for Si = 0, 1, 2, 4, 8, 16) using this constant are

[1] Using the approximation formula given by Fisher, *loc. cit.* p. 199.

6·159, 5·066, 7·526, 8·705, 11·041, 10·585. The greater part of the variance in the no-manure series is thus accounted for, but the variance of deviations remains high and in comparison with the very low value of the variance of parallels is fully significant. The variance of deviations (5 degrees of freedom) is 2·2628; the variance of parallels (18 degrees of freedom) is 0·56305. Thus $z = \cdot69564$ while for a probability of ·05, z is about 0·51. The chief divergence between the expected and the observed values lies in the absence from this series of the depression in yield with one unit of silicate shown by the 5 manures containing both nitrogen and potash. For the purpose of fitting the general equation for the silicate-phosphate interaction this depression was accepted as part of the data from which constants for the effect of the different doses of silicate were to be derived. Tested statistically the mean depression in yield for unit dose of silicate is just on the verge of significance. For Si = 0 the mean yield for pots with N and K and varying P is 12·9558; for the same manures with Si = 1 unit the mean yield is 11·1252. The mean difference is 1·8306 while the level of significance ($\sigma m_1 - m_2 \times t$ for a probability of ·05) for this comparison is very little greater—1·99.

It is just possible therefore that the apparent depression in yield with unit dose of silicate may not be real. In fact the error in representing the mean value of E (Table of Relative Efficiencies, p. 77) as a linear function of amount of silicate is, up to Si = 8, less than the error due to differential response. Beyond Si = 8, there may be a depression or not: the data again are inadequate for an exact decision.

Up to Si = 8 units however a reasonable account of the results in terms of concentration of superphosphate and concentration of silicate may be obtained from an equation of the type we have been discussing:

$$Y \text{ (Yield)} = \frac{1}{k_1 + \frac{k_2}{(p+P)E}},$$

where $k_1 = \cdot02922$ for manuring with N and K,
$= \cdot031404$ for manuring without K,
$= \cdot03837$ for manuring without N or K,

$k_2 = \cdot0503$, $p = \cdot406$,

P = gm. of superphosphate supplied as manure and E (representing the efficiency of the superphosphate) = $\cdot8403 + \cdot192 \times$ Si, where Si = no. of units of silicate supplied.

This involves six constants derived from the yield data. For the variance

of deviations of the 35 yields up to Si = 8 we have therefore 29 degrees of freedom and the variance is 82·7097/29 = 2·85206. The variance of parallels (105 degrees of freedom) is 2·10324. Hence $z = 0{\cdot}15228$, while the value of z required for a probability of ·05 is about ·227.

Thus within the limits set by the variation of parallel pots it seems possible to formulate the effect of added silicate in terms of an increase in the efficiency of the superphosphate present, this increase in efficiency approximating to a linear function of amount of silicate up to 8 units of silicate.

D. Summary.

(1) Under controlled conditions in water cultures soluble silicate was found to have little effect upon the growth of barley if phosphorus were also present, but if the latter were absent a significant increase in dry weight was induced by the silicate.

(2) The addition of silicate caused an appreciable increase in the height of the main shoot, which was most marked in phosphate-free solutions, becoming less evident as the quantity of phosphate present was increased.

(3) Leaf development was retarded by phosphate deficiency and hastened by the addition of silicate.

(4) A close association exists between the amount of phosphate present, and the effect of silicate upon the rate of tillering and the number of tillers developed.

(5) Soil cultures with barley and mustard in pots with various forms of silicate showed that soluble silicates are more active than glass silicates, tending to cause increase in dry weight with deficient mineral manuring, and in some cases also with complete manuring.

(6) Further soil experiments revealed variations in the response of barley and mustard to silicate on different types of soil. A general improvement occurred with increasing doses of silicate together with various combinations of manures, notably when phosphorus or potash was deficient.

(7) The significance of the results obtained has been examined statistically, and an attempt made to formulate the effect of added silicate in terms of an increase in the efficiency of the superphosphate present.

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EXPLANATION OF PLATE V

Fig. 1. Barley grown in nutrient solution containing no phosphate, with sodium silicate ranging from 0–16 units (1st harvest).

Fig. 2. Barley grown in complete nutrient solution, with sodium silicate ranging from 0–4 units (1st harvest).

Fig. 3. Barley grown on light soil with sulphate of ammonia and superphosphate, but no potash, with sodium silicate ranging from 0–16 units, left to right.

Fig. 4. Mustard grown on light soil with sulphate of ammonia and potash but no phosphate, with sodium silicate ranging from 0–16 units, left to right.

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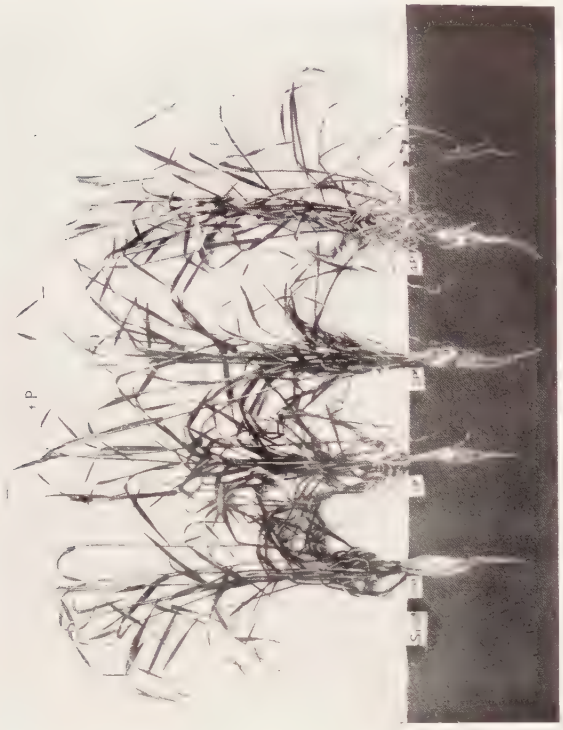

Fig. 2.

Fig. 4.

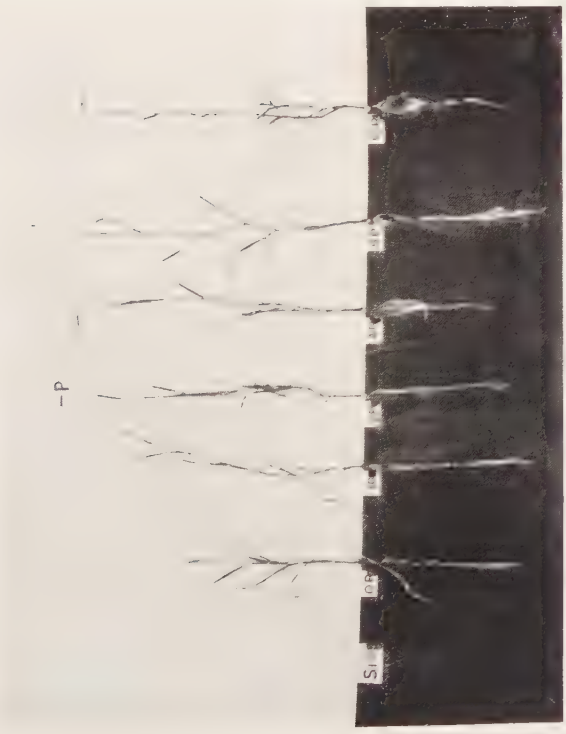

Fig. 1.

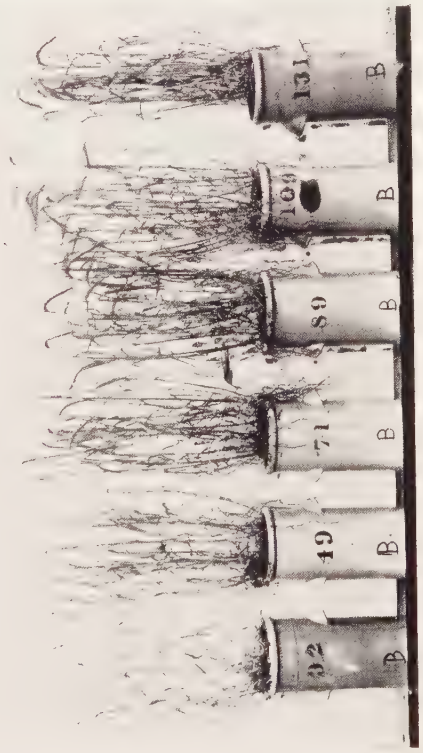

Fig. 3.

BRENCHLEY, MASKELL & WARINGTON.—Silicon and other Elements in Plant Nutrition (pp. 45–82).

THE INFLUENCE OF *TILLETIA TRITICI* (BJERK.) WINT. AND *TILLETIA LAEVIS* KÜHN ON THE GROWTH OF CERTAIN WHEAT VARIETIES

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(With Plates VI and VII and 1 Text-figure.)

CONTENTS.

I. Introduction.

In a recent series of experiments dealing with the control of bunt in wheat by the use of copper dusts and other chemicals unexpectedly high increases in yield were obtained from the treated grain in which bunt was reduced to a minimum, as compared with the untreated plots in which varying percentages of bunt occurred (12)[1]. Analysis of the data revealed the fact that rows sown with treated grain produced in nearly every experiment a greater number of heads at harvest than rows of grain not treated, although both lots had been sown at a uniform seed rate (*loc. cit.* Table IX). Further evidence of the superiority of the treated seed was obtained in three experiments by a study of early growth in the field, January to May, when the rows from treated seed were conspicuous for their thicker stand and more vigorous development of shoots.

In these experiments bunt-free and untreated seed of the same variety was not available for comparison, therefore it was impossible to

[1] A similar result was recorded by Heggi(7) in 1911, who found that in attacks of bunt the reduction in yield did not correspond to the percentage of diseased spikes.

discriminate between the effect of eliminating bunt and other influences which the chemical as such might possibly have on the growth of the plant.

In addition to the superior early growth of untreated rows, it appeared on a superficial examination that healthy plants were longer in the straw than those carrying bunted heads, and since little attention had previously been directed to the influence of the fungus on the growth of the plant, the authors decided to make a critical comparison of the establishment, tillering, growth in height and final yield of healthy and bunted plants of the same variety of wheat, reserving as a distinct and separate problem the influence of treatment with chemicals.

The problem was studied during the years 1924–5 by three methods:

(1) Establishment and yield data were obtained from rows of bunt-free and bunt-contaminated grain, sown under field conditions in the cage at the Plant Breeding Station Farm. (Experiment I.)

(2) Germination and dry-weight data were collected from seedling plants grown in boxes under varied conditions of soil and temperature. (Experiment II.)

(3) Measurements were made throughout the entire growth period on single plants, bunted and healthy. The plants in this case were grown in pots in the cage at the Plant Breeding Station Gardens. (Experiments III and IV.)

The results prove that bunt has a retarding influence on growth in height, which under certain conditions manifests itself at an early stage in the development of the plant. Tillering, on the other hand, appears to be stimulated by the parasite, bunted plants showing on the average a higher number of tillers per plant than those which are healthy. This result is of particular interest in view of the data from field trials previously referred to, in which treated rows carried a far higher number of heads at maturity than those not treated, but the apparent discrepancy in results may be explained by the failure, under certain conditions, of bunt-contaminated seed to establish a good stand in the field, a result obtained with three varieties in 1925. (Experiment III.)

It is evident that bunt has a more retarding effect on growth and yield than has been generally realised, and the remarkably high increase in grain yield following its control in field experiments can now be explained without resort to the hypothesis of a stimulating effect on the part of chemicals applied to the grain. There is, however, still the possibility that treatment does also influence growth in this way, or in preventing the attacks of soil organisms during the germination period,

and the question of the effect of various chemicals on the establishment and growth of bunt-free wheat samples is the subject of a separate investigation. Data on the growth of grain treated with copper carbonate are, however, given in one experiment.

II. Historical.

The early investigations on bunt of wheat, prior to the year 1921, have been summarised by Woolman and Humphrey (16), and it is noteworthy that little attention has been given to the effect of the fungus on the host plant apart from the final production of spores in place of healthy grain.

That bunt has a retarding influence on the development of the wheat plant was noted by Lang (8) in 1917. A dwarfing effect due to *T. tritici* was described by Harwood (4) and other workers (11) and it was suggested that this character might be made use of for distinguishing the two species *T. tritici* and *T. laevis* in the field since the latter fungus appeared to influence the growth of the plant only to a very slight extent.

Various references have been made to morphological differences between the ears of healthy and bunted plants. A lengthening was recorded for ears of the squarehead (2) and *compactum* types (6, 9, 15) but in a spring bearded wheat no difference in length was apparent between healthy and bunted heads (13). Some measurements on ears and kernels, healthy and bunted, were given by Barrus (1), but the first worker to record data estimating the influence of bunt on growth in height was Mourashkinski in 1925 (10). Both species of *Tilletia* were included in his experiments, which consisted of field plots of bunt-free and bunt-contaminated samples of the same variety sown at spaced intervals and left until maturity when they were taken up for measurement. The data show conclusively that the infected plants were shorter in each internode than plants raised from healthy seed, while plants with healthy ears raised from contaminated seed occupied an intermediate position.

In 1924, using *Triticum vulgare* var. *milturum*, the depressing effect of the two species of *Tilletia* was equal, but in 1925 it was slightly more marked in the case of *T. tritici*, although the same pure line of wheat was used as in the previous year. It is suggested that the difference between the two sets of data may possibly be attributed to the fact that growth conditions were not so favourable for wheat in 1924, or perhaps to a difference in the spore collections of *T. laevis* used in the two seasons.

In several experiments counts were made in the field of seedlings established in plots sown respectively with healthy and bunt-contaminated grain. Under some conditions it was found that the mortality was distinctly higher in the case of bunted grain, but the degree of mortality varied with the variety and with the time and conditions of sowing the grain. A higher percentage of mortality was actually found in the case of certain varieties recorded on the basis of plants with bunted heads as less susceptible.

Discussing the actual cause of mortality among bunted plants, the author refers to the fact that such plants are more than ordinarily susceptible to attacks of *Fusarium* sp.—a fungus causing considerable damage to wheat in the east of Russia.

In addition to a shortening of the stem internodes, bunted plants showed in the majority of varieties a reduction in the length of ear and in the number of spikelets produced, but three varieties gave contrary results. Where such reduction did occur it was manifest also in healthy ears raised from contaminated seed.

Finally, data are given on the size of bunted grain, and a certain correlation is found to exist between this character and the susceptibility of a variety to bunt, the bunted grains being longer than the normal in the more susceptible varieties and shorter than the normal in those that are more resistant.

The experiments described below carried out on somewhat different lines and with different wheat varieties confirm in all essential points the results obtained in Russia. In addition, data are given on the progressive tillering of bunted and healthy plants, and on the influence of *Tilletia tritici* on the height and the dry weight of bunted and healthy plants in the seedling stage.

III. Experimental Data.

(1) Establishment and yield data from rows of bunt-free and bunt-contaminated grain sown under field conditions. (Experiment I.)

Varieties: Browick, Marshal Foch, Svalöf Iron. Sown February 18th, 1925.
April Bearded, Red Marvel. Sown April 9th, 1925.

Replications: Eight 5-ft. rows of each treatment for each variety.

The object of this experiment was two-fold: (i) to compare the behaviour of healthy and bunted grain of the same variety grown under exactly similar conditions; (ii) to study the effect of the copper carbonate treatment on grain free from bunt.

Since sufficiently large samples of grain of known origin were not available, it was necessary to obtain new samples from a seed firm. Of the five varieties included in the experiment, four were apparently bunt-free, in that the grain sown without treatment did not produce a single bunted head. The fifth sample, April Bearded, was evidently bunt-contaminated on receipt, the control rows containing at harvest an average of 9·7 per cent. bunted heads.

The five varieties received uniform treatment, except in regard to the dates of sowing, which varied with the winter and spring varieties.

Each bulk sample (1 lb.) was divided into four lots which received the following treatment:

A. Control—grain as received.

B. Grain as received, shaken with copper carbonate at the rate of 2 oz. per bushel.

C. Grain as received, shaken with bunt spores at the rate of 1 gm. spores per 100 gm. grain. The spores were derived from the variety April Bearded in 1924, the bunted grain being kept in a tin out-of-doors from October to February, then crushed and sieved.

D. Half of the contaminated sample (C) was treated with copper carbonate at the same rate as that used for the healthy grain, sample (B).

The varieties were sown at a uniform rate, 120 grains per 5-ft. row and each treated lot was replicated eight times.

The data collected from the four varieties in which the control rows were entirely bunt-free, are given in Table I, while those obtained from April Bearded are shown separately in Table II.

Discussion of Results.

The winter varieties sown in February were subject to highly unfavourable climatic conditions during the first seven weeks, with the result that germination was extremely slow and uneven[1]. When critically examined in May a marked difference was evident between the rows, the control of each winter variety having made a distinctly better stand than the bunt-contaminated lots. Rows from grain treated with copper carbonate (B) were not quite so good as the control (A), but rather better than those in which the copper dust had been applied to bunt-

[1] The following data were kindly supplied by Mr Martin G. Jones, M.Sc., meteorological observer at the Welsh Plant Breeding Station: For the week ending March 1st, 1925, the total rainfall for the week was 2·14 in., some rain fell each day and the minimum temperature ranged from 32 to 39° F. During the first seven weeks after sowing (February 18th, 1925) twenty-one days showed ground frost and rain fell on twenty-eight days.

contaminated grain (D). The rows were graded on a system of marking in which a maximum of five marks was allocated to a thick uniform row and two days later actual counts were made on each row in the experiment, the two sets of data showing close agreement. (Table I.)

The two spring varieties sown early in April had already germinated and made a uniform stand when the first count was made, no difference appreciable to the eye being evident between the rows. In actual figures the lots treated with copper carbonate showed in both varieties slightly better establishment than the control or the bunt-contaminated rows.

Comparing the healthy control (A) rows with those containing bunted heads in regard to the number of plants established in May, the number found at maturity and the average number of heads per row at harvest, a progressive increase in the influence of bunt is evident, amounting to 16, 19 and 25 per cent. for the three sets of data in the case of lots badly bunted (B); 14, 15 and 17 per cent. in the case of lots where bunt was partially controlled by the copper dust treatment (D). (Table I.)

The retarding influence of bunt on growth in height was especially conspicuous at the "boot stage," just prior to emergence of the spike. Measurements were made in each row at three points, situated approximately in the middle, and at a foot from the two ends. The distance measured in the one case was from ground level to the tip, and in the other, to the ligule of the flag leaf of the tallest tiller at each place of measurement. The results show for each variety an appreciable reduction in the height of bunted rows, although the measurements were not made exclusively on plants showing bunted heads since such plants could not be recognised at that stage of growth.

Considering the grain yield data we find here also a confirmation of the results of previous field experiments. Taking the average of four varieties the percentage loss in grain weight is 64, while the percentage bunted heads is 57. The difference between the two figures is no doubt due to the lower rate of establishment of the bunt-contaminated grain.

It is not the purpose of the present paper to treat fully of the influence of chemicals on the growth of wheat, but reference might be made in this connection to the series (A) and (B), which show in all the data collected remarkably close agreement. From this experiment, therefore, it cannot be said that the copper dust has definitely affected either beneficially or adversely the healthy grain, whereas the retarding influence of bunt is seen at all stages in the growth period.

A further point of interest in the experiment is the relatively high percentages of bunt obtained in the lots treated with copper carbonate,

Table I.

Showing establishment, height and yield of four wheat varieties, bunted and non-bunted, treated with copper carbonate. Winter varieties sown February 18th, 1925. Spring varieties sown April 9th, 1925. Knoll cage.

Treatment and variety	Bunted plants %	Bunted heads %	Marks for braird-ing. Max. 5 20. v. 25	No. of plants estab-lished 22. v. 25	No. of plants at maturity average per row	Height to tip of flag leaf 16. vi. 25 cm.	Height to ligule 26. vi. 25 cm.	No. of heads per row at maturity	Total weight straw+ grain gm.	Weight of healthy grain gm.	Yield of healthy grain: control at 100
A. Control: grain as received:											
Browick	0·0	0·0	2·0	19·2	25·1	59·3	54·8	58·9	310·8	92·8	100
Marshal Foch	0·0	0·0	2·8	21·4	27·4	59·9	57·0	62·9	325·8	117·3	100
Svalöf Iron	0·0	0·0	3·8	39·5	54·8	57·6	51·3	80·1	358·5	129·6	100
Red Marvel	0·0	0·0	5·0	57·4	58·2	57·8	56·6	81·8	269·8	81·3	100
Average	0·0	0·0	3·4	34·4	41·4	58·7	54·9	70·9	316·2	105·2	100
B. Copper carbonate on grain as received:											
Browick	0·4	0·2	1·8	18·4	23·5	57·1	52·7	57·3	294·9	85·2	92
Marshal Foch	0·0	0·0	2·1	19·9	26·0	61·5	56·4	65·8	329·6	122·4	104
Svalöf Iron	0·0	0·0	3·4	36·1	44·1	57·1	52·5	74·4	325·6	113·6	88
Red Marvel	0·8	1·0	5·0	62·5	67·0	58·4	56·4	98·8	306·9	94·2	116
Average	0·3	0·4	3·1	34·2	40·2	58·5	54·5	74·1	314·2	103·9	100
C. Contaminated with bunt spores:											
Browick	53·1	42·6	1·1	12·1	15·1	53·2	46·8	36·8	169·6	42·1	45
Marshal Foch	43·9	43·1	1·6	15·2	22·4	53·0	48'2	42·4	177·2	54·8	47
Svalöf Iron	54·9	51·3	2·5	29·5	36·6	52·4	46·0	51·8	185·2	53·9	42
Red Marvel	92·7	91·4	5·0	58·6	58·4	52·3	49·3	80·8	162·2	7·8	10
Average	61·2	57·1	2·6	28·9	33·1	52·7	47·5	52·9	173·6	39·7	36
D. Contaminated with bunt spores and treated with copper carbonate:											
Browick	8·0	4·2	1·5	13·9	17·8	57·3	51·6	45·0	261·6	79·7	86
Marshal Foch	10·1	6·1	2·1	15·4	22·3	62·3	54·4	52·4	254·4	88·0	75
Svalöf Iron	13·8	12·8	2·4	25·6	32·4	53·2	54·4	54·8	241·6	85·3	66
Red Marvel	41·6	38·4	5·0	63·4	68·2	57·8	56·8	84·5	237·4	62·5	76
Average	18·4	15·4	2·9	29·6	35·2	57·7	54·3	59·2	198·8	78·9	76

Table II.

Showing data on establishment, growth and yield of April Bearded wheat with varying amounts of bunt. Sown April 9th. 1925. Knoll cage.

Treatment	Bunted heads at harvest %	Av. number of plants per row 22. v. 25	Av. height to tip of flag leaf 16. vi. 25 cm.	Av. height to highest ligule 26. vi. 25 cm.	Av. number heads per row at harvest	Total weight straw+ grain gm.	Weight o healthy grain gm.
Grain as received	9·7	66·1	83·1	65·6	112·4	323·8	95·8
Copper carbonate on grain as received	0·9	71·5	75·5	67·3	127·9	371·9	114·3
Contaminated with bunt spores	93·1	66·4	64·2	57·3	112·5	193·0	7·0
Contaminated and treated with copper carbonate	52·7	72·0	72·5	63·6	131·9	313·2	57·6

but it should be understood that the rate of spore contamination, 1 per cent., was excessively heavy and one hardly like to occur under practical conditions[1]. This may also account in part for the unusually high percentage of bunt obtained with spring-sown wheat which is commonly held to be less heavily attacked than wheat sown in the autumn.

(2) Germination and dry weight data obtained from seedling plants under varied conditions of soil and temperature.

Variety: Marshal Foch. Sown November 17th, 1925. (Experiment II.)

For this experiment a clean bulk sample was divided into two portions, one of which was kept as the control, while the other was shaken with spores of *T. tritici*, known to be viable. The grain was sown at spaced intervals in boxes measuring 21 × 12 × 7 in. and covered to a uniform depth. Each box carried 104 grains. In half of the total number of boxes (36) partially sterilised soil was used, the remainder were filled with soil which had not been sterilised[2]. The boxes were divided between three places of experiment, a heated glass-house, a cold glass-house and an open cage, in order that the plants might be subject to different conditions of temperature. The experiment thus included twelve series, each consisting of three replications. All the boxes were sown and placed in position on the same day. Notes and counts on the rate of germination were made at intervals, and in the cold house, where a decided difference in height was apparent between the "healthy" and "bunted" series, the individual plants in each box were measured from soil level to the apex of the longest leaf. Dry weight data were finally obtained by carefully removing the plants from each box and separating shoots from roots by cutting the hypocotyl at the point where the grain was still attached. Except in the case of those situated in the heated house, which were the first to be taken up, the shoots were dried and weighed in lots of twenty plants, and the weight per 100 was calculated on this basis. The roots were also washed, dried and weighed, but the data have been omitted from Table III as the experimental error was relatively high and no definite conclusion could be drawn from the results.

[1] In experiments conducted by Heald (5) a definite relation was found to exist between the spore load carried by the grain and the percentage disease appearing in the crop. Maximum smutting was produced when 0·5 gm. of spores were applied per 100 gm. grain.

[2] Partial sterilisation was achieved by heating the soil in a brick oven. The soil was first saturated with water and heated to 85–90° C., this temperature being maintained for approximately 12 hours.

Table III.

Showing a comparison between the early growth of plants raised from bunt-free (control) and bunt-contaminated grain and sown under varied conditions of soil and temperature. Variety: Marshal Foch. Sown November 17th, 1925.

Place of experiment	Age of seedlings when taken up weeks	Germination in soil. %		Av. dry weight of shoots per box in gm.		Av. dry weight of shoots per 100 plants in gm.		Av. height per plant in cm.	
		Control	Bunt-contaminated	Control	Bunt-contaminated	Control	Bunt-contaminated	Control	Bunt-contaminated
Partially sterilised soil:									
Heated glass-house	8	92	90	5·853	4·960	6·096 (100)	5·276 (87)	—	—
Cold glass-house	17	98	98	7·305	6·049	7·141±·076 (100)	6·002±·021 (84)	22·2±·110 (100)	18·5±·137 (83)
Open cage	21	97	95	17·154	17·173	16·940±·243 (100)	17·149±·213 (101)	—	—
Non-sterilised soil:									
Heated glass-house	8	88	92	4·230	4·380	4·640 (100)	4·560 (98)	—	—
Cold glass-house	17	78	73	4·632	3·465	5·732±·108 (100)	4·558±·097 (80)	18·8±·176 (100)	16·2±·221 (86)
Open cage	21	80	80	4·866	5·816	5·912±·249 (100)	6·920±·271 (117)	—	—

Discussion of Results.

The different temperature conditions to which the plants were subject are indicated by the rate of germination in the three places of experiment. In the heated glass-house an average of 22 plants per box were above soil level on November 26th, only nine days after sowing, and the maximum establishment figure was obtained on December 11th. On this date germination in the cold glass-house was only just apparent, while in the open cage not a single seedling was in view. In this situation no plants appeared above soil for five weeks from the date of sowing. During this period the temperature fell below freezing point on nineteen days and the actual range for the same period was maximum 36–45° F., minimum 18–40° F.

In regard to the percentage germination no constant difference was apparent between the bunt-contaminated grain and the bunt-free control. The greatest difference was a 5 per cent. decrease in the cold house (non-sterilised soil), but in the same soil, in the heated house, a 4 per cent. increase was recorded for the same grain. Taking the average of all boxes in the three places of experiment the results are 94 and 96 per cent. for bunt-contaminated and bunt-free grain respectively in the partially

sterilised soil and in the non-sterilised soil 82 per cent. germination was recorded for both lots of grain.

In later growth, however, there was a distinct and visible difference between the control and the bunt-contaminated grain in both soils in the cold house as is shown by Plate VI (2) and (3).

By measurement the difference in height in favour of the control plants was found to amount to 14 and 17 per cent. for the non-sterilised and partially sterilised soils respectively. A distinct and significant difference in the same direction was obtained in the dry weight of shoots per 100 plants. In non-sterilised soil the control shoots were 20 per cent., in partially sterilised soil 16 per cent. heavier than those derived from bunt-contaminated grain.

In the heated house, and in the open cage, no visible difference could be detected between the two lots of seedlings in either type of soil. Plate VI (1) and (4) shows the similarity of the two series in height and general growth, and it was not thought useful to make actual measurements. In regard to the dry weight of shoots per 100 plants a 13 per cent. decrease was shown by the plants raised from bunt-contaminated grain in partially sterilised soil, but from the range of figures it is doubtful if the difference is significant, and it was not confirmed in the other type of soil.

In the open cage again no reliable difference was evident. Apart from the low temperature the plants here were subject to extremely severe conditions of wind and rain, and this increased the accidental differences between boxes in the same series. The apparently large difference (17 per cent.) in favour of the bunted lots in the dry weight of shoots per 100 plants is without mathematical significance.

In this experiment, therefore, a retarding effect due to bunt was distinctly shown at an early growth stage under one set of conditions, but was absent when the same seed was grown under two different sets of conditions.

A somewhat similar result was obtained in another experiment. It was noticed in February in a field trial that rows of seedlings raised from bunt-contaminated grain treated with copper carbonate were far superior in appearance to others of the same seed not treated. Since samples of both lots of seed were still available, 416 grains from each sample were sown in boxes which were placed in the heated glass-house. Dry weight data were obtained after eight weeks as in the above experiment, but no appreciable difference in shoot development existed between the two lots, thus giving a result contrary to that obtained under field conditions.

A slight difference between the two lots was, however, shown in the root data:

	Dry weight shoots only		Dry weight roots only	
	Average	Range	Average	Range
Bunt-contaminated grain	2·665	(2·09–3·87)	0·333	(0·29–0·39)
Grain treated with copper carbonate	2·655	(2·31–2·96)	0·392	(0·33–0·49)

It is possible that the similar behaviour of the two samples in the heated house was due to the fact that the fungus failed to infect the plants under the temperature conditions there prevailing, since it is well known that a low temperature favours infection of wheat by this fungus (3). Unfortunately this point could not be tested by growing on the plants to maturity. On the other hand, in experiments described below the retarding influence of bunt on growth was evident at later growth stages, but inappreciable in the young plant, and its absence at that stage does not of necessity mean that plants are free from infection.

It has not been possible to investigate more fully this apparent correlation between temperature conditions and the influence of bunt on growth. Reference might be made to the previous section of this paper in which it was shown that the establishment of bunt-contaminated grain was lowered in the case of three varieties sown in February while the effect was absent in the case of two other varieties sown in April. Mourashkinski recorded the fact that bunt-infected plants may die before maturity and discussing the problem states that the percentage of death varied with several factors, including the time of sowing.

(3) Measurements made throughout the entire growth period on single plants, raised from bunt-contaminated and bunt-free grain.

Varieties: Hen Gymro, pure line selection. Sown March 14th, 1924. (Experiment III.)
April Bearded. Sown March 4th, 1925. (Experiment IV.)

The method adopted with the variety Hen Gymro in 1924 was briefly as follows. The bulk sample of grain was first sterilised by soaking it for ten minutes in formalin solution (1 part in 320 parts of water) and covering it for four hours with a cloth soaked in the same solution. The grain was then thoroughly washed with tap water and finally spread out to dry. Before sowing, the sample was separated into grain of two sizes, and the average weight of each lot was determined. For the experiment each grain sown was weighed separately and only those were included which showed a difference of less than 12 per cent. from

the average weight of the grain in the particular series. Each lot of selected grain was further subdivided, one half being reserved for the control, and the other half contaminated by shaking with spores of *Tilletia tritici*. The experiment thus consisted of four series:

(1) Small grain bunt-free (control) ... ... 8 pots
(2) „ „ bunt-contaminated ... ... 17 „
(3) Large „ bunt-free (control) ... ... 8 „
(4) „ „ bunt-contaminated ... ... 17 „

Each series was sown on March 14th, 1924, four grains being allowed for one pot (tomato size) with the intention of allowing three to reach maturity. In a few instances it was necessary to make up the number in a particular pot by transplanting seedlings from one pot to another in the same series. This was carefully done at a very early stage of growth without any apparent check on the growth of the plant.

Measurements on height were made at weekly intervals during the growing season and at the same time counts were made on the tillers produced by each plant.

At maturity each plant was taken up, wrapped round with paper and classified, after examination in the laboratory, as healthy, semi-bunted or completely bunted, according to the condition of the grain. Only those plants were placed in the last group when the heads failed to show a single healthy grain. Data were also obtained for each plant on the number of heads, the weight of straw and the height of each tiller

Table IV.

Showing the relative tillering and height of single plants of wheat raised from bunt-free and bunt-contaminated grain. Variety: Pure line selection Hen Gymro. Sown March 14th, 1924.

Variety	No. of plants averaged	No. of heads at maturity per single plant	Height to base of ear cm.	Height to apex of ear cm.	Length of ear (by difference) cm.	Weight of straw per plant gm.
Small grain. Contaminated (*T. tritici*):						
Not-bunted	10	7·8	103·9	115·2	11·3	17·2
Semi-bunted	21	7·0	89·7	100·2	10·5	12·3
Completely-bunted	12	8·9	80·2	89·8	9·6	11·9
Control	23	7·7	98·8	109·4	10·6	16·9
Large grain. Contaminated (*T. tritici*):						
Not-bunted	8	6·9	102·3	114·6	12·3	15·4
Semi-bunted	28	8·7	91·5	103·1	11·6	18·0
Completely-bunted	11	8·7	95·6	94·8	9·2	14·9
Control	23	7·5	102·5	115·3	13·2	16·7

measured from the level of the root system to the base and the apex of the ear. The results are given in Table IV.

The experiment was repeated in the following year, using April Bearded as the variety of wheat, and including the species *Tilletia laevis* in addition to *T. tritici*[1]. The method of carrying out the experiment was essentially the same as in the previous season with one or two slight modifications. The sample was graded as before, but only grain of one "size" was used, namely that which fell between ·025–·035 gm. in weight. Heated soil was used as a few plants had been lost in 1924 by wireworm attack, and six grains were sown per pot to guard against the necessity of transplanting. These were finally thinned to four as it was found that such a number could reach maturity without becoming pot-bound. The experiment thus consisted of the following series:

(1) Bunt-free seed (control) ... ... 10 pots.
(2) Seed contaminated with spores of *T. tritici* ... 20 ,,
(3) ,, ,, *T. laevis* ... 10 ,,

As in the previous experiment, data on tillering and growth in height were collected during the growing season and at maturity the plants were carefully lifted and tied in paper. In addition to measurements on length of straw, number and weight of heads and grain, dry weight data were obtained on the roots, which were carefully freed from soil and then washed and dried. The root system was severed by cutting across the base of the plant at a point level with the highest root on the stem. The results are given in Table V.

Discussion of Results.

In the 1924 experiment, using Hen Gymro wheat, the "small" grain gave 77 per cent., the "large" grain 83 per cent. of bunted plants. In regard to height of straw and length of ear the average figures for plants raised from the large-sized grain were slightly higher than those obtained by sowing the smaller seed, but as far as the influence of bunt on growth is concerned the two series gave closely parallel results. Thus it is evident (Table IV) that in both series bunted plants when compared with the control showed a decided reduction both in height (16–19 per cent.) and in the length of the ear (9·4–30·3 per cent.). The reduction was, however, less in plants not completely bunted.

In 1925 a similar reduction (amounting to 13 per cent.) in the length of straw was obtained with *T. tritici*, on April Bearded wheat, but no appreciable difference was apparent in the length of ear, the average

[1] Spore collection of *T. laevis* was derived from the variety Poole. It was placed at the authors' disposal by the kindness of Dr G. H. Pethybridge.

for bunted plants being very slightly higher than that for the control. The diseased plants in this experiment showed in almost every case a certain number of healthy grain, and therefore no classification could be made into groups of semi-bunted and completely-bunted individuals.

Table V.

The relative growth and yield of single plants of wheat raised from bunt-free and bunt-contaminated grain. Variety: April Bearded. Sown March 4th, 1925.

Treatment	No. of plants averaged	No. of ears per plant at maturity	Height of base of ear cm.	Height to apex of ear cm.	Length of ear (by difference) cm.	Weight of single complete plant excluding roots gm.
1. Grain contaminated with spores (*Tilletia tritici*). Bunted plants	62	9·5 ±·215	71·28 ±·886	81·06 ±·952	9·78	24·84 ±·716
2. Grain contaminated with spores (*Tilletia laevis*). Bunted plants	24	9·16 ±·358	79·90 ±1·03	90·20 ±1·05	10·30	26·60 ±·914
3. Control	36	8·70 ±·245	81·90 ±·840	92·20 ±·869	9·70	28·70 ±·816
4. Apparently healthy plants from (1). (*T. tritici*)	9	8·40 ±·481	87·69 ±1·426	98·51 ±1·377	10·82	31·78 ±1·386
5. Apparently healthy plants from (2). (*T. laevis*)	16	9·12 ±·259	82·30 ±1·30	93·80 ±1·49	11·50	30·30 ±·972

Treatment	Weight of ears per single plant gm.	Weight of grain per single plant gm.	No. of grain per single plant	Weight of healthy grain per plant gm.	Weight of roots only gm.	Weight of straw only gm.
1. Grain contaminated with spores (*Tilletia tritici*). Bunted plants	10·05	6·45	309	3·85	1·62 ±·055	14·79
2. Grain contaminated with spores (*Tilletia laevis*). Bunted plants	10·63	6·86	254	5·34	2·10 ±·111	15·97
3. Control	13·45	9·16	260	9·16	2·09 ±·105	15·25
4. Apparently healthy plants from (1). (*T. tritici*)	14·05	10·03	269	10·03	2·14 ±·136	17·73
5. Apparently healthy plants from (2). (*T. laevis*)	14·20	10·02	252	10·02	2·04 ±·098	16·10

When partially-bunted plants are considered, the influence of the fungus is shown in the following average figures obtained by classifying the heads as healthy, semi-bunted and completely-bunted. Where the infection of the head was complete the height was reduced by 15 and 19 per cent. in the two series, but where the infection was partial the height varied from apparently healthy tillers by only 1 and 7 per cent. In these plants, therefore, the height of individual tillers shows the same correlation with the degree of infection as the average height of tillers on plants when these are classified on the same basis with the single plant as the unit.

Table VI.

Showing the average height of tillers from plants partially bunted. Hen Gymro, 1924.

Series	Healthy ears cm.	Semi-bunted ears cm.	Completely-bunted ears cm.
Small grain	96·7	95·7	82·0
Large „	104·5	97·2	84·4

The weight of straw is clearly a function of the number of tillers at maturity and their height. In these experiments, with the exception of the semi-bunted plants in the large-grain series 1924, the bunted units, in spite of their superiority in the number of tillers fall slightly below the control plants in weight of straw.

As has been stated, measurements on height were made throughout the growing season at weekly intervals. For the first month (1925) no constant difference between the bunted series and the control was obtained, the bunted (*T. tritici*) leading slightly in measurements of the first and third tillers, while the reverse condition held in the case of the second tiller. An indication that bunted plants were falling behind the control in growth appeared in the measurements obtained in May and June, which were as follows:

Average height in cm. of healthy and bunted plants.

	May 27th	June 3rd	June 10th	June 17th
Healthy	51	64	79	88
Bunted (*T. tritici*)	49	62	78	85

It is obvious that with such a small average difference bunted plants could not be detected by the eye on this character[1].

While dealing with the retarding influence of the fungus on growth it is interesting to find that in 1925 where data were obtained on the weight of roots, a significant difference amounting to 23 per cent. was shown in favour of the control plants as compared with those infected by *T. tritici*.

Turning now to the question of tillering, the fungus has apparently the reverse effect to that recorded for growth in height. In 1924 with both series of plants the healthy controls showed fewer heads at harvest than plants which were completely bunted, the difference being approxi-

[1] It was necessary to obtain measurements on each plant in the series and re-group them at a later date when the plants could be classified on the presence and absence of bunted grain in the ear.

mately 14 per cent. in each case (Table IV). In 1925 bunted plants (*T. tritici*) again showed increased tillering as compared with the control (Table V). At harvest the differences amounted to only 8·4 per cent. as compared with 14 per cent. with Hen Gymro in the previous experiment, but the difference was more striking when the plants were compared at the period of maximum tiller development. In Fig. 1 the relative tillering of the three series of plants is shown graphically for the complete growing period. In this character the two species of *Tilletia* showed a

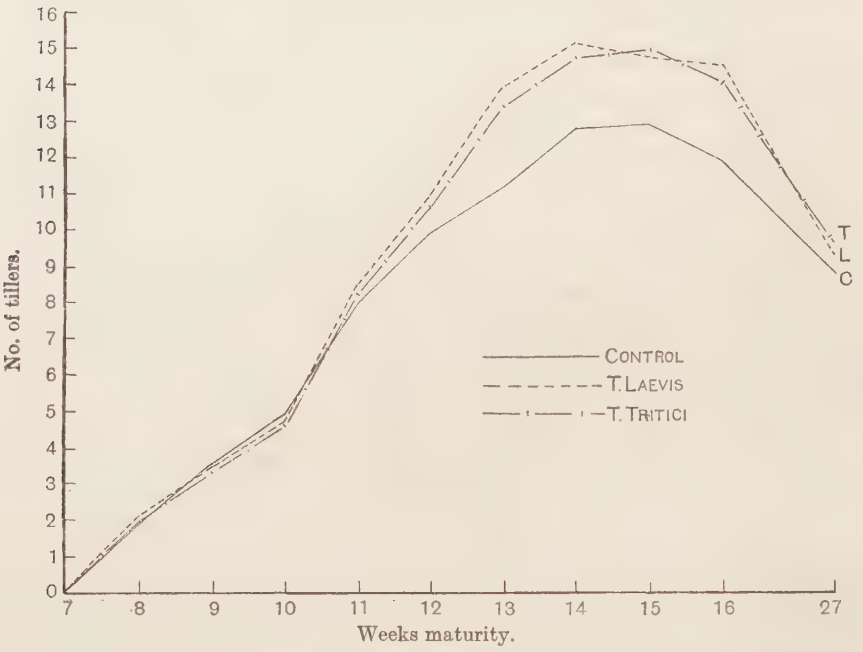

Fig. 1. Graph showing the average number of tillers per plant at weekly intervals. Healthy and bunted plants. Variety: April Bearded. Sown March 4th, 1925.

remarkably close parallel. In the fourteenth week when the maximum number of tillers was recorded the bunted plants of both series showed an increase of 15 and 17 per cent. respectively over the control.

In another experiment in which the plants were grown in rows under field conditions a similar difference in tillering was recorded between bunted and healthy plants, although owing to the influence of competition the actual number of heads produced at harvest was much smaller than in the pot experiments here described. The figures for this experiment are as follows:

Table VII.

Showing relative tillering of healthy and bunted plants grown in rows under field conditions, 1924.

Variety	Number of plants examined	Total number of heads at harvest	Average number of heads per plant
Hen Gymro, 24 pure line selections:			
Infected (*T. tritici*)	566	2007	3·55
Healthy	269	820	3·05
Twelve other varieties (*T. vulgare*):			
Infected (*T. tritici*)	74	233	3·15
Healthy	163	473	2·90

Before leaving the question of growth and yield, reference should be made to the data obtained in this connection from plants derived from bunt-contaminated seed which failed to show any diseased grain in the ear, and were classed, therefore, as "apparently healthy." In both seasons' experiments such plants were found to agree more closely as regards height, length of ears and weight of roots (one season) with the healthy plants in the control series than with the plants obviously bunted. The same is true also in regard to tillering with the exception of the *T. laevis* series in 1925. In these experiments the plants gave in most cases even slightly higher figures than the healthy control plants, suggesting that they had perhaps escaped or grown away from the disease by reason of their extra vigour. The number of such plants which came under investigation is, however, too small to admit of reliable conclusions on this point. In certain experiments conducted in Russia (10) "apparently healthy" plants raised from contaminated seed were found to occupy a position intermediate in regard to height between healthy control and obviously bunted plants.

Except where special reference has been made to the tillering of plants affected by *T. laevis* in the 1925 experiment, the above conclusions refer to the species *T. tritici*. From Table V it is evident that the plants affected by *T. laevis* show in their length of straw, weight of straw and weight of roots, only slight differences when compared with healthy control plants. In regard to height the difference though slight is in the same direction as that shown by the plants infected with *T. tritici*. That the retarding influence of *T. laevis* on growth in height is considerably weaker than that of *T. tritici* has been stated by Potter and Coons (11) in America, and a similar result was obtained in one set of experiments by Mourashkinski (10).

Special attention was paid in these experiments to the possibility of distinguishing bunted and healthy plants in the early growth stages, particularly as it has been stated that infected plants are more luxuriant in growth and darker in colour. The first point appears to be true in so far as the average tillering capacity is concerned, but this feature was not sufficiently marked to make it possible to identify with certainty bunted plants without examination of the ears. The writers failed also to detect any constant variation in the colour-shade of the vegetative organs, but a distinct difference in colour was apparent in the ears, especially towards maturity. Healthy ears changed from green to yellow before those that were diseased, and there came a period when the bunted plants were conspicuous by reason of the dark or blue-green colour of the pales and glumes. At an earlier stage of development the infected plants could only be recognised by opening the sheath and examining the young ovaries. As described by Barrus (1) the ovary of bunted plants is then considerably swollen and bright green in colour, the stamens are short and the anthers greatly reduced in size. No exsertion of anthers takes place from bunted flowers, a fact which serves as a further distinguishing mark of infected plants when the ear has emerged from the sheath.

The white appearance and the spreading position of the glumes in ripe bunted ears is as familiar as the rounded form of the bunted grains. In some ears affected by *T. laevis* abnormally long grains were found, in some cases stretching as far as 0·5 cm. beyond the pales. On closer examination it was found that the shape of the grain could not be used as a reliable means of distinguishing the two species of *Tilletia* and it was necessary to resort to the microscope and to determine the character of the spore wall.

IV. Summary and Conclusions.

Summarising the results of the experiments described in the present paper, it is evident that bunt has a distinct influence on vegetative organs of the plant in addition to the well-known effect on the grain. Both species which cause bunt, *Tilletia tritici* and *T. laevis*, were included in one experiment only, from which it appears that the influence of the latter species, while tending in the same direction, was distinctly weaker in its effect than *T. tritici*. This conclusion is in harmony with the results of other workers (4, 10, 11).

The following conclusions refer to *T. tritici*, with which species the work was mainly carried out:

(1) *Soil germination and establishment.*

The actual percentage of germination has not been found to differ appreciably in bunt-free and bunt-contaminated samples, but in one experiment a considerable difference was manifest in the final establishment, the bunt-free samples showing an increase of 25–37 per cent. when compared with those which were contaminated with spores before sowing (Table I). The greatest difference (37 per cent.) was shown by the variety Browick, which gave the lowest establishment figure and had presumably suffered most intensely from the unfavourable climatic conditions.

(2) *Early growth.*

The first visible symptoms of a retarding influence on growth are not always manifest at the same stage of development in the wheat plant. Under one set of conditions (p. 92) the effect was clearly shown by plants in the seedling stage, since those derived from bunt-contaminated seed differed from the control by 14–17 per cent. in height, and 16–20 per cent. in the dry weight of 100 shoots. In another experiment (p. 97) where a considerable reduction in height was evident at maturity the plants showed no appreciable effect during the first ten weeks of growth.

(3) *Tillering.*

In the matter of tillering the influence of the fungus was in the opposite direction to that recorded for growth in height, bunted plants at the period of maximum tiller development producing in one experiment 16 per cent. more tillers than healthy plants grown under precisely the same conditions. At maturity in both cases the number of heads was considerably less than the total number of tillers produced, but bunted plants still showed an increase over those that were healthy (Experiments III and IV).

A similar difference was noted in the case of plants grown in rows in the field[1].

(4) *Length of straw at maturity.*

A reduction in the length of straw appears to be one of the most constant results of the influence of the fungus on vegetative growth. It will no doubt be shown that the degree of reduction varies with the variety, the season and possibly with the origin of the spores used for contamination. In the experiments here described a reduction in height

[1] Increased tillering and reduced height have been recently described in oat plants affected by loose smut (*Ustilago avenae*) (14).

amounting to 16–19 per cent. in the case of Hen Gymro and 13 per cent. in the case of April Bearded was recorded. It was shown moreover that the reduction was considerably greater in the case of tillers carrying heads in which every grain was bunted than in the case of those bearing both healthy and bunted grain.

(5) *Length of ripe ear.*

In the case of Hen Gymro wheat the fungus showed a slight retarding effect on the length of the ear, while in the variety April Bearded the bunted ears were, if anything, slightly longer than the normal. More data are desirable on this point, since the influence on this organ is probably largely determined by the morphology of the ear in any particular variety. It appears to be generally recognised that ears of the *compactum* type are abnormally narrow and long when bearing bunted grain (6, 15). Plate VII shows bunted and normal ears of *T. vulgare* var. Standard Red, which possesses normally relatively wide, dense ears. For comparison are shown healthy and diseased heads of Hen Gymro, which has ears characteristically lax.

(6) *Root development.*

In one experiment where data were obtained on the dry weight per 100 plants of the roots at maturity a significant difference was apparent between bunted and healthy specimens, the former showing a decrease of 22 per cent.

From the practical point of view, where the final yield of healthy grain is of primary consideration, the most important aspect of the question, apart from the effect on the grain itself, is probably the influence of the disease on establishment and early growth. It is of course recognised that any check which the plant receives in the early stages of growth will be exaggerated later in the case of plants placed in competition with healthy individuals, as must happen in a partially bunted field crop. The full extent of the retarding influence on growth was possibly not exhibited in certain of the experiments under review since the bunted plants were not seriously subject to competition.

Since it has been shown that the presence of the parasite tends to increase tillering, the reduction in heads at harvest which was a striking feature of previous treatment experiments must be attributed either to the stimulating effect of the chemical used for treated lots, or to the depressing influence of bunt on establishment in the field. From the data here given the second alternative seems the more feasible, but since the influence of bunt on establishment is, perhaps more than any other,

likely to be affected by seasonal conditions and to differ with the variety and the origin of the seed, it is desirable to obtain further data on this aspect of the problem, using a large number of seed samples and varieties and a wide range of environmental conditions.

V. Acknowledgments.

The authors desire to express their most cordial thanks to Professor R. G. Stapledon for placing at their disposal the facilities of the Welsh Plant Breeding Station, and for his helpful interest in the work. They desire also to thank Mr J. W. Watkins for his careful supervision of cultural details. The authors are indebted also to Dr E. J. Butler and to Dr G. H. Pethybridge for references to papers bearing on the subject of this paper.

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EXPLANATION OF PLATES VI AND VII

PLATE VI.

Seedlings raised from bunt-contaminated and bunt-free grain of the same variety—Marshal Foch—sown November 17th, 1925. The bunted sample is on the left in each case.

(1) Heated glass-house, seedlings 8 weeks old, partially sterilised soil.
(2) Cold glass-house, seedlings 16 weeks old, partially sterilised soil.
(3) Cold glass-house, seedlings 16 weeks old, non-sterilised soil.
(4) Open cage, seedlings 16 weeks old, non-sterilised soil.

A distinct difference in height was shown in both types of soil between the bunted and healthy plants raised in the cold house (2) and (3).

PLATE VII.

(1) Bunted and healthy ears of *T. vulgare*, variety Standard Red.
(2) Bunted (*B*), healthy (*H*) plants of *T. vulgare*, variety Hen Gymro derived from contaminated grain.
(3) Healthy and bunted ears of *T. vulgare*, variety Hen Gymro.

(*Received August 9th*, 1926.)

Fig. 1.

Fig. 2.

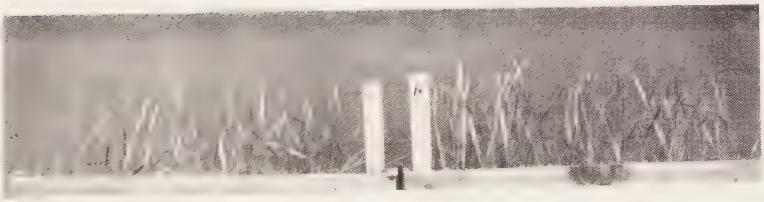

Fig. 3.

Fig. 4.

SAMPSON & WALTERS DAVIES.—On the Growth of certain Wheat Varieties (pp. 83–104).

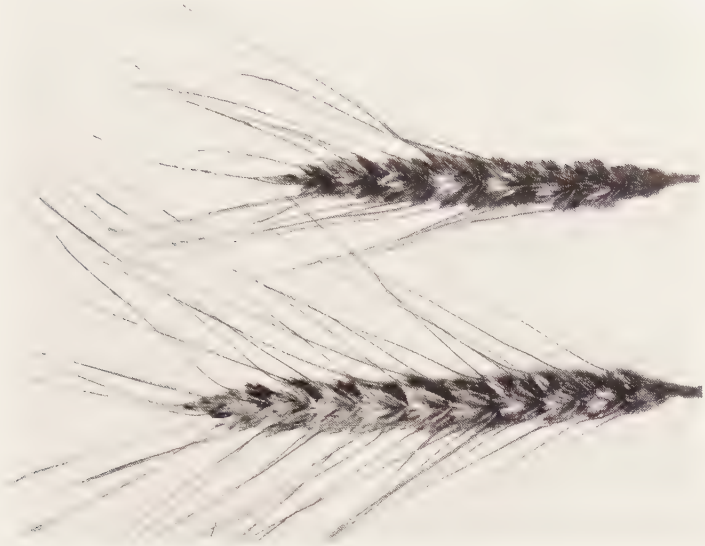

Fig. 3.

Fig. 2.

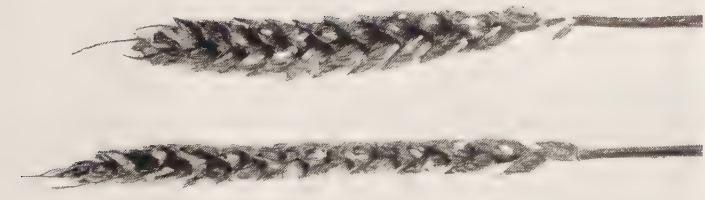

Fig. 1.

SAMPSON & WALTERS DAVIES.—On the Growth of certain Wheat Varieties (pp. 83–104).

THE INCIDENCE AND INTENSITY OF *PUCCINIA GLUMARUM* ERIKS. AND HENN., ON WHEAT INFECTED AND NON-INFECTED WITH *TILLETIA TRITICI* WINTER, SHOWING AN APPARENT RELATIONSHIP BETWEEN THE SUSCEPTIBILITY OF WHEAT PLANTS TO YELLOW RUST AND TO BUNT

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(*School of Agriculture, Cambridge.*)

(With 1 Diagram.)

It is the custom on the University Farm, Cambridge, to carry out various experiments on the control of the bunt fungus, *Tilletia tritici*. The following note records an interesting relationship, observed during the past season 1925–6, between yellow rust of wheat, *Puccinia glumarum*, and bunt, or stinking smut of wheat, *Tilletia tritici*. The wheat in the main block of experiments was sown in October 1925. In May it was observed that certain plots were more yellow than others, and that the yellowing was largely due to a severe attack of yellow rust. Later, when it was possible to identify the plants that were infected with bunt, the plots were more critically examined and it was seen that the bunted plants were all very badly rusted. Since the variety of wheat on which detailed observations were kept was Little Joss, this correlation between bunt and yellow rust was interesting. Little Joss wheat is a cross between Square Head's Master which is neither markedly resistant nor markedly susceptible to yellow rust, and a Russian wheat, Ghirka, which is only slightly susceptible. The degree of rust resistance of this hybrid, Little Joss, compares with the rust resistance of the Ghirka parent.

It is often noticed, however, that in a year when yellow rust is severe Little Joss wheat becomes infected quite early in the season; later, as the plants develop, they "grow away" from the disease and when the plants are in ear only an occasional pustule is found upon the leaves and this generally does not break through the epidermis.

Supporting the evidence of this apparent relationship between bunted and rusted plants there is an isolated observation by Armstrong (1) which,

of direct value, confirms the conclusions which have been drawn from the experiments. The following quotations are selected from that paper:

Page 87: "The two extracted rust-resistant types and American Club continued to show very high resistance to attack, but in a few cases this resistance had apparently been more or less broken down. These cases will be noticed after first comparing the number of such apparent "breakdowns" on the different beds."

Page 88: "On bed B (row 2), 1 plant was slightly flecked, and another bore a few unbroken pustules on one blade. In row 10 the 4 plants remained free from infection. In row 6 there were only 2 plants; one of these had 4 leaves badly attacked, and on these over 200 pustules were counted, many of which were freely shedding spores. Some weeks later it was discovered that this plant was also infected with bunt (*Tilletia caries*)."

On the same page there appears the following as a footnote: "Since then another similar case has been observed. In an F_3 culture (from the cross Wilhelmina and American Club) raised from an immune F_2 plant all except one plant were rust-free. This odd plant was very severely attacked by rust, *and was also found to be infected with bunt.*"

In one series of experiments wheat had been sown weekly from October 1925 to March 1926, each plot being of six rows, a row being 16 ft. This wheat had been contaminated prior to sowing with "bunt balls" obtained from Little Joss wheat grown in 1925. It was contaminated at the rate of 1 part by weight of "bunt balls" to 25 parts of weight of wheat; from this stock the requisite amount of wheat was weighed out and sown weekly. On July 1st 500 leaves were picked at random from each of these 24 plots. These leaves were examined in the laboratory for the presence of rust and recorded as having none, slight, moderate or severe. A leaf was recorded as severely rusted if the whole or $\frac{3}{4}$ of its surface was completely yellow with rust pustules, moderate if $\frac{1}{4}$ to below $\frac{3}{4}$ rusted, slight if from a few scattered pustules or streaks to below $\frac{1}{4}$ rusted. In such a crude classification the main difficulty is in determining whether a leaf is, or is not attacked, since with this variety there are peculiar fleckings on the leaf, and, on these, pustules of yellow rust may, or may not be present. Since the proportion of slight to none, on this account, was thought to be of little value as an indication of intensity of attack, the number of leaves showing a moderate attack was added to the number showing a severe attack. The total, expressed as a percentage of the whole sample (500 leaves) was taken, in what follows, as the "Intensity of yellow rust attack." Table I shows

the values of intensity of rust attack for the series of weekly sowings of bunt-infected "seeds."

At a later date the percentage of bunted ears in these plots was

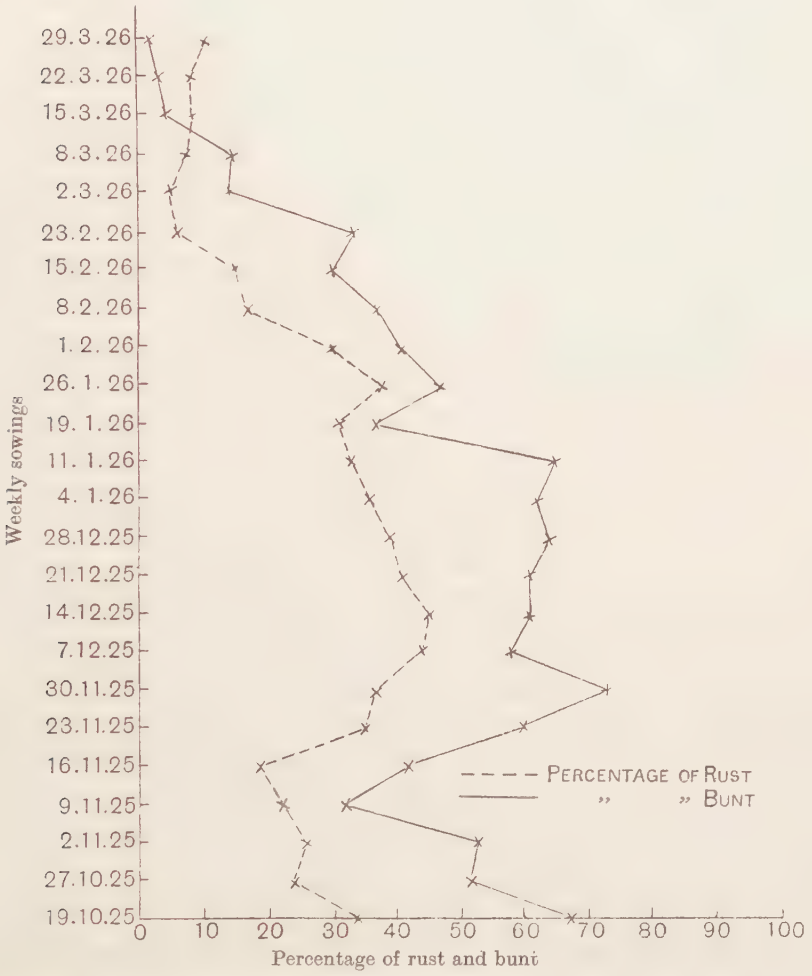

Diagram I. Showing a correlation between the intensity of a yellow rust attack and the percentage of bunt in experimental plots of wheat artifically contaminated at a uniform rate (1·25) with bunt balls and sown weekly from 19. 10. 25 to 29. 3. 26.

estimated by taking a count of 1000 ears from a diagonal band across every plot.

In Diagram 1 the percentage of bunt and the percentage of rust are plotted against the date of sowing. The general trend of values suggests

Table I.

Showing the intensity of yellow rust attack, and the percentage of bunt, on Little Joss wheat sown weekly from October 19th, 1925 to March 29th, 1926.

Date of sowing	Intensity of yellow rust attack	Percentage of bunt	Date of sowing	Intensity of yellow rust attack	Percentage of bunt
19. x. 25	33·6	68·2	11. i. 26	32·8	65·2
27. x. 25	23·6	51·9	19. i. 26	31·2	37·4
2. xi. 25	26·4	52·8	26. i. 26	38·4	47·1
9. xi. 25	22	31·8	1. ii. 26	30·2	40·8
16. xi. 25	18·8	41·6	8. ii. 6	17	36·8
23. xi. 25	34·8	60·3	15. ii. 26	15·4	30·4
30. xi. 25	37·3	71·5	23. ii. 26	6	32·9
7. xii. 25	44	58·1	2. iii. 26	5	14·0
14. xii. 25	44·6	61·3	8. iii. 26	7	15·4
21. xii. 25	40·6	60·5	15. iii. 26	7·8	3·9
28. xii. 25	38·8	63·6	22. iii. 26	8·4	3·1
4. i. 26	35·6	61·5	29. iii. 26	9·8	1·0

a definite association between the incidence of rust and bunt in this experiment. It may be, however, that this connection between bunt and rust is spurious. This is to say, it can be argued that, since this wheat was sown on 24 different dates, it was offered 24 different environments, and that these were variably favourable to *both* the diseases and *that* is the true reason why the relationship between their incidence shows up. From the evidence in Tables II and III this is unlikely since in Table II, Plot 1, the percentage of bunted ears is 90 and the lowest percentge of rust is 72. On Plot 4 in a count of 1000 ears there was no bunt and the highest percentage of rust is 2·6. Now Plot 4 was sown within an hour of Plot 1 on the same day. Consider Plots V to XII; these also were sown within a short time of each other on the same day, yet as the percentage of bunted ears increases with the heavier contamination of "bunt balls," so does the percentage of yellow rust increase; and, as the copper carbonate treatment reduces the percentage of bunted ears, so is the percentage of yellow rust reduced. It is a logical conclusion to assume a definite correlation between the two diseases.

In a second series of experiments, and these were the most convincing, Little Joss wheat was contaminated with different amounts of "bunt balls" and sown on the same date. The percentage of bunt and the intensity of rust attack were estimated in the way already described, but where 100 leaves only were examined the uppermost leaf of the stem was observed. These estimates are recorded in Table II. They show

Table II.

Showing a relationship between intensity of yellow rust and the percentage of bunt in plots of Little Joss wheat which have been contaminated at varying rates with "bunt balls."

Plot number and treatment	Date of observation	Total No. of leaves examined	Percentage of yellow rust						Percentage of bunt
			None	Slight	Moderate	Severe	None + Slight	Moderate + Severe	
I									
Untreated (1 part by weight of bunt balls to 25 parts by weight of wheat)	20. vi. 26	100	11	17	—	—	28	72	90
	24. vi. 26	1993	8·2	6	—	—	14·2	85·8	
	13. vii. 26	100	10	8	13	69	18	82	
II									
Copper carbonate dusted 3 oz. to the bushel. Contamination 1 : 25	20. vi. 26	100	75	25	0	0	100	0	10·2
	13. vii. 26	100	51	28	5	16	79	21	
III									
Steeped copper sulphate 2½ %. Contamination 1 : 25	20. vi. 26	100	56	43	1	0	99	1	2·5
	13. vii. 26	100	74	25	1	0	99	1	
IV									
Steeped formaldehyde 1 : 240. Contamination 1 : 25	20. vi. 26	100	55	44	1	0	99	1	0
	24. vi. 26	1554	92·3	5·1	—	—	97·4	2·6	
	13. vii. 26	100	84	16	0	0	100	0	
V									
Dusted copper carbonate 3 oz. per bushel. Contamination 1 : 25	14. vii. 26	100	43	36	1	20	79	21	11·5
VI									
Untreated. Contamination 1 : 25	20. vi. 26	100	17	10	—	—	27	73	94
	24. vi. 26	512	9·9	1·8	—	—	11·7	88·3	
	5. vii. 26	500	10·2	13·8	33·8	42·2	24	76	
VII									
Dusted copper carbonate 3 oz. per bushel. Contamination 1 : 50	14. vii. 26	100	45	49	3	3	94	6	5
VIII									
Untreated. Contamination 1 : 50	20. vi. 26	100	24	17	—		41	59	78
	24. vi. 26	485	21	7	—	—	28	72	
	5. vii. 26	500	22	12·8	25·2	40	34·8	65·2	
	14. vii. 26	100	18	30	7	45	48	52	
IX									
Dusted copper carbonate 3 oz. per bushel. Contamination 1 : 100	14. vii. 26	100	52	47	0	1	99	1	3
X									
Untreated. Contamination 1 : 100	20. vi. 26	100	36	33	—	—	69	31	49·7
	24. vi. 26	360	30·2	9·5	—	—	39·7	60·3	
	14. vii. 26	100	45	25	25	5	70	30	
	5. vii. 26	500	31·2	25	25·2	18·6	56·2	43·8	
XI									
Dusted copper carbonate 3 oz. per bushel. Contamination 1 : 500	14. vii. 26	100	22	69	7	2	91	9	0·9
XII									
Untreated. Contamination 1 : 500	20. vi. 26	100	73	19	—	—	92	8	25
	24. vi. 26	377	52·8	11·7	—	—	64·5	35·5	
	5. vii. 26	499	43·1	45·1	9·2	2·6	88·2	12·8	
	14. vii. 26	100	44	21	8	27	65	35	
XIII									
Untreated. Contamination 1 : 25	13. vii. 26	100	7	10	15	68	17	83	92 5
LVIII									
A clean sample of seed (?) sown in non-infected (?) ground	24. vi. 26	216	60·6	36·1	—	—	96·7	3·3	1
	8. vii. 26	499	19·6	66·5	12·7	1·2	86·1	13·9	
	13. vii. 26	100	47	52	1	0	99	1	
XXXII									
Untreated. Contamination 1 : 25	8. vii. 26	500	13	37·4	31·4	18·2	50·4	49·6	86·2
	10. vii. 26	500	13·8	26·8	34·8	24·6	40·6	59·4	

Table III.

Showing intensity of yellow rust on varieties o wheat infected and non-infected with bunt.

Variety	A Yellow rust intensity in 100 bunted tillers	B Yellow rust intensity in 100 tillers free from bunt
Wilhelmina	100	2
Rector	100	35
Benefactor	98	48
Square Head's Master	100	10
Victor...	98	6
Marshal Foch ...	100	8
Iron	100	0
Yeoman	90	22
Little Joss	100	0
Rivet*	90	0
Red Marvel	93	35

* Intensity slight, not strictly comparable with intensity in the other varieties.

Table IV.

Showing the intensity of rust attack on the first four leaves of each of 100 *tillers of Rivet wheat,* 57 *having bunted ears, the remainder being free from bunt.*

Intensity of rust attack	Uppermost leaf	Second leaf	Third leaf	Fourth leaf
None	40	40	36	17
Slight	10	12	9	11
Moderate	48	46	55	68
Severe	2	2	1	4

a relationship between the intensity of yellow rust and the percentage of bunt in plots of Little Joss wheat which has been treated in various ways and has previously been contaminated at varying rates with "bunt balls." In the last series to be described in this note various English varieties of wheat were contaminated at the rate of 1 part by weight of "bunt balls" to 25 parts by weight of wheat, sown, and observed for bunt and rust.

These observations are in Table III; they show the intensity of yellow rust on wheat plants, affected and non-affected with bunt. In recording the observations tillers were taken at random throughout the plot; if the tiller had a bunted ear it was recorded in column A, if it was free from bunt it was recorded in column B. Since this was a field examination, in estimating the intensity of rust, the uppermost leaf was examined, and if showing a slight (+), moderate or severe attack was described as

rusted. Rivet wheat appeared the least susceptible to yellow rust. Previous to the above observations on Rivet wheat this same variety was examined on July 10th. One hundred tillers at random were uprooted and examined leaf by leaf and the intensity of rust recorded. These estimates are in Table IV; they show the intensity of rust attack on the first four leaves of each of the 100 tillers of Rivet wheat, 57 of which had bunted ears, the remainder being free from bunt.

In Table III there is a further possibility of spurious relationship, for it is possible that the tillers which were both rusted and bunted were the very late ones, and it is possible that mere lateness made them prone to both diseases. From the above evidence and from general eye impressions on badly and slightly bunted plots it appeared to the writer that this was not the true explanation, but that in the season 1925–6 there was a very close correlation between yellow rust and bunt, insomuch as bunted plants were definitely more rusted than plants free from bunt; and, in the case under consideration, it was evident that infection of Little Joss wheat with the bunt fungus was, in effect, capable of breaking down the resistance that Little Joss has, in its later stages, to *Puccinia glumarum*.

The authors (2) of Research Monograph No. 4, Ministry of Agriculture and Fisheries, describe how the F_3 and F_4 cultures of the cross between Wilhelmina and American Club were made use of to test the possibility of breaking down the resistance to yellow rust by manuring excessively with soluble nitrogenous manures. They describe how various "doses" were given to the cultures, the maximum corresponding to the rate of some eight hundredweight of nitrate of soda per acre. They state that, "though the season was unfavourable for rust, and comparatively little was to be found on the other wheat plots, the susceptible cultures were severely attacked whilst those raised from resistant F_2 plants, though examined leaf by leaf from the late spring until the foliage began to die off, remained free, with the exception of an occasional pustule which did not break through the epidermis." On the evidence that is produced below it appears to the writer that rust resistance which cannot be broken down artificially by the use of soluble nitrogenous manures may be broken down by natural contamination of seed wheat with the bunt fungus. It is suggested that Little Joss, to be completely resistant to yellow rust, would need to be resistant to certain other fungoid diseases of which *Tilletia tritici* is one. The converse, however, that a wheat variety immune to bunt must be immune to yellow rust does not follow, since wheat varieties resistant to bunt that we have had under observation for two years have been badly rusted.

SUMMARY.

In the season 1925–6 an interesting relationship was noticed between yellow rust of wheat, *Puccinia glumarum* and bunt or stinking smut of wheat, *Tilletia tritici*; it was observed that:

1. Bunted Little Joss wheat plants were badly rusted and that plants free from bunt were free or comparatively free from rust.

2. Bunted plants of other wheat varieties were definitely more rusted than plants free from bunt.

3. It is suggested that rust resistance which cannot be broken down artificially may be broken down by natural contamination of wheat with the bunt fungus.

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(*Received August 21st*, 1926.)

OBSERVATIONS ON THE INSECT CARRIERS OF MOSAIC DISEASE OF THE POTATO.

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(*The University, Manchester.*)

(With Plates VIII–X and 1 Text-figure.)

CONTENTS.

1. Introduction.

The observations recorded in the following pages are from experiments upon mosaic disease of potatoes carried out during the three or four years previous to the end of 1925 and deal with the part played by insects in its dissemination.

The first and most important step was definitely to ascertain which, if any, of the insect fauna of the potato plant were instrumental in transmitting this virus disease, and this was the object of the experiments here detailed; such points as the optimum condition of plant and insect for infection, the actual organ or organs in the body of the insect which contain the virus and the possibility of inherited infectivity in the insect, etc., are left for future study.

The writer is indebted to Dr George H. Pethybridge for kindly criticising the ms. Acknowledgments are also due to Dr James Davidson for suggestions regarding the insect-proof cages, to Mr H. Britten for taking some of the photographs, to Dr T. Whitehead for supplying some of the mosaic-infected potato tubers, and to Mr Theobald and Dr C. L. Walton for supplying some of the aphides.

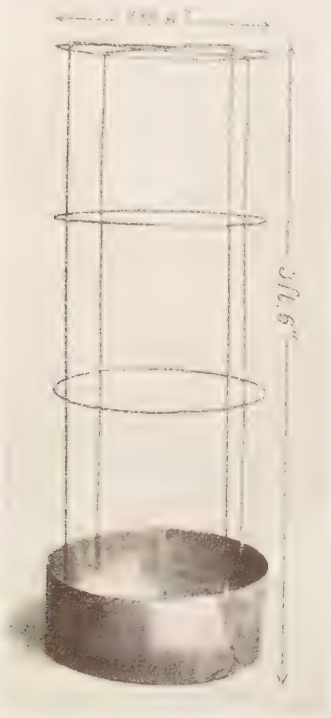
2. Technique.

In view of the great difficulty experienced by workers on virus diseases of the potato in obtaining an absolutely insect-proof, and especially aphis-proof frame, it may not be out of place to describe the various types of cage used in these experiments prior to the adoption of the frame now in successful use.

Two types of wooden frame covered with fine quality muslin of twisted linen thread were first used, the earlier type made of inch square laths, $2\frac{1}{2}$ ft. high by 4 ft. long. This was soon discarded for the second type of wooden frame which was 4 ft. high by 6 ft. long and was divided into three compartments, each compartment holding one plant, screened from its neighbour by a canvas partition. A small trap door gave access to the plant. The bottom of the frame was provided with a strong tarred base which enabled the whole structure to be sunk in the ground to a depth of 6 in.; the frame was covered with a strong linen net. Neither of these wooden frames proved very efficient, aphides gaining entrance in a number of cases. Probably the ill-success of these frames was due more to the failure of the material covering them, than to any defects in the construction of the frame itself. Many kinds of material, both metal and fabric were tried, but all succumbed to the unusual acidity of the Manchester atmosphere and it was not until the frame, now in use, was evolved that a really aphis-proof structure could be found. The next attempt at an insect-proof cage consisted of a large glass cylinder covered with a canvas top which was placed over the growing plant. This proved to be effective so far as excluding aphides was concerned, but the conditions prevailing inside the cylinder were such as to "draw" the plant unduly. These cylinders proved however to be of the greatest use for breeding upon infected potato plants the insects, which were later to be used as carriers of the virus.

Finally, a cage which was insect-proof and which yet allowed the plant to grow fairly normally, was obtained. This is a modification of one used by Davidson in his aphis work at Rothamsted, and is illustrated in Text-fig. 1. It consists of a circular framework made of brass wire, 18 in. in diameter and 3 ft. 6 in. high. The base of the frame is fitted with a cylinder of heavy galvanised iron, which allows the structure to be sunk to a depth of 6 in. in the soil. Over the frame is fitted a cylindrical covering made of strong unbleached calico, and this is doubly bound with tarred twine round the top of the iron base to prevent slipping. It was found necessary to use brass wire for the frame-

work, as iron wire, even if tinned over, rusted through the canvas. This cage was used successfully for the transmission experiments in 1925. In 1926 it was further improved and will be described in the account of



Text-fig. 1. The brass framework of the cages used in the 1925 experiments 3 ft. 6 in. in height and 1 ft. 6 in. in diameter. The cylindrical iron base which is sunk into the ground is shown.

that year's experiments. One disadvantage of this cage is that it has to be lifted bodily to gain access to the plant within, thus exposing the latter to the danger of chance infection by flying aphides. but with a little practice this can be avoided.

3. Description of Experiments.

The inoculations described in these preliminary experiments were of two kinds; that is, efforts were made to induce the various insects to inoculate a potato plant with mosaic disease (*a*) by feeding on the haulm.

(*b*) by feeding upon the sprouts of the tuber. In both cases the insects used were infected, or presumably infected, with the mosaic virus by being bred upon a "mosaic" potato plant inside one of the glass cylinders previously described.

It may be worth while briefly to outline the experiments prior to the year 1925, which, although negative in their results, were yet carefully carried out and should possess some value.

Experiments in years 1922–4.

(*a*) Attempted transmission of mosaic by feeding infected insects upon the haulms of known healthy potatoes. In these experiments six of the large wooden frames were used and two single frames, and lettered *A* to *H*. The potatoes used were Great Scot and the source of infection was a number of Golden Wonder plants affected with mosaic in a mild form. The insects used were the aphides *Myzus persicae*, *Myzus circumflexus*, and the capsid bug *Lygus pabulinus*.

Frame *A*, with three compartments, contained three plants, each from one quarter of the same tuber of Great Scot, while a fourth plant from the fourth quarter was isolated in a single frame *G*. Infected individuals of *Myzus persicae* were then introduced into compartments 1 and 3 of the frame while the plant in compartment 2 and the plant in frame *G* acted as controls.

Frame *B* was treated similarly, except that *Lygus pabulinus* was substituted for the aphis, and the plant from the fourth quarter was isolated in a single frame *H*. The other four frames *C*, *D*, *E*, *F*, each with three compartments were treated in much the same way, using *M. circumflexus* and *M. persicae*. In these, however, the tubers were cut into three parts, the plants arising from each one-third acting as controls to the two experimental plants from the two other thirds respectively.

The results of this experiment, so far as it was carried, were entirely negative, both controls and experimental plants remaining healthy throughout the season. Owing to illness the writer was unable to save the tubers from these plants and grow them the following year; had this been done it is possible that some disease might have made itself evident.

(*b*) The following sprout inoculations with aphides were also carried out in the same year as the above:

(1) On May 11th, 1922, a number of adult females of *Macrosiphum gei* (= *solanifolii*) were transferred to the sprouts of six half-tubers of healthy Great Scot and placed in aphis-proof glass vessels. These aphides

had been breeding for 21 days upon a plant of Great Scot which was affected with mosaic disease.

A similar number of adult females of *M. gei* from a known healthy Great Scot plant were transferred to the sprouts of the six remaining half-tubers of the healthy Great Scot and placed also in glass containers; these six halves acted as controls to the preceding ones.

(2) The above experiment was repeated, using however another species of aphis (*Myzus circumflexus*). The source of infection this time was a mosaic Golden Wonder, and the six control half-tubers were not fed upon by any aphides from a healthy plant. On May 24th, thirteen days later, the half-tubers were cleared of aphides and planted. The twenty-four plants resulting proved healthy and remained so throughout the summer.

Experiments in 1923.

In 1923 another short series of sprout inoculations by means of aphides was carried out, this time using the species *Myzus persicae* and *Macrosiphum gei*. The following is a brief description of these inoculations.

(*a*) On May 18th, twelve adults of *M. gei*, which had been feeding for fourteen days upon a mosaic Great Scot, were placed six each upon two halves of Rhoderick Dhu tubers, the other two halves, untouched by insects, acting as controls. On May 23rd the aphides were removed and the four half-tubers planted.

(*b*) On May 16th, twelve individuals of *M. gei*, from a mosaic Golden Wonder, were placed upon the sprouts of two half-tubers of Rhoderick Dhu, the other two halves acting as controls. The times of planting, etc., were as in (*a*).

(*c*) The same as (*b*) but using *Myzus persicae* in the place of *Macrosiphum gei* and substituting Great Scot for Rhoderick Dhu.

(*d*) In this experiment *Myzus persicae* from infected Great Scot were transferred to half-tubers of Edzell Blue. One half-tuber only used in this experiment.

(*e*) *M. persicae* from mosaic Golden Wonder to half-tubers of Arran Chief. Times and numbers the same as (*a*), (*b*) and (*c*).

Results of 1923 *sprout inoculations.*

All the plants resulting from the half-tubers, both experimental and control, remained healthy throughout 1923. The following point of interest arises: in the experiment (*d*), it was noticed that the source of

mosaic infection, viz. the mosaic Great Scot (used in (*d*) only) upon which *M. persicae* had been fed, was also showing signs of leaf-roll. Both plants, however, arising from the half-tubers—the inoculated and the control—which were grown under cover remained healthy, but on growing the progeny of these two plants the following year, out of a total of twelve tubers in (*d*) all gave leaf-roll plants without mosaic, and of the ten tubers of (d_1) (the control) all were healthy. Conclusions cannot be based upon this isolated occurrence, but the suggestion might be made that *M. persicae* had transmitted leaf-roll to the exclusion of mosaic.

On April 25th, 1924, the tubers resulting from the 1923 sprout inoculations were planted with the following results:

(*a*) Six experimental tubers gave all healthy plants; seven control tubers gave all healthy plants.

(*b*) Five experimental tubers gave all healthy plants; five control tubers gave all healthy plants.

(*c*) Six experimental tubers gave all healthy plants; four control tubers gave all healthy plants.

(*d*) Twelve experimental tubers gave leaf-roll plants; ten control tubers gave healthy plants.

(*e*) Six experimental tubers gave all healthy plants; three control tubers gave healthy plants.

This concludes the account of the attempts made up to the end of 1924 to induce insects to transmit mosaic disease of the potato. It is clear that the evidence of such transmission is almost entirely negative, and although aphides under certain circumstances may transmit potato mosaic disease, it seems certain that under other circumstances even when transmission might well be expected to occur, it does not do so.

In 1925 the insect transmission work was restarted on a wider scale, with an improved technique in the shape of reliable insect-proof frames (Plate VIII, fig. 1), and extended to include all the usual Hemipterous insect fauna of the potato plant. These insects have already been carefully studied in regard to their methods of feeding (9) upon the potato plant, and certain theories have been put forward as to their capabilities as disease carriers deduced from this study of their feeding methods. It is hoped that these theories may be confirmed or disproved by the inoculation studies now to be described.

Description of 1925 experiments.

The following insects were used in the 1925 experiments:

HEMIPTERA.

HETEROPTERA.

Capsidae.	*Calocoris bipunctatus.*
	Lygus pabulinus.

HOMOPTERA.

Typhlocybidae.	*Zygina pallidifrons.*
	Eupteryx auratus.
Aleurodidae.	*Asterochiton vaporariorum.*
Aphididae.	*Myzus persicae.*
	Myzus circumflexus.
	Macrosiphum (solanifolii) gei.

The work was divided into two parts: (*a*) the placing of infected insects on the shoots of sprouting half tubers; (*b*) the placing on the haulms of growing healthy plants of insects previously infected as a result of feeding upon mosaic diseased potato plants. In order to avoid introducing a possible complicating factor, the same variety of potato was used as far as possible for the source of insect infection and for the experimental transmission. This variety was President, the source of infection being plants affected with mild mosaic and the potatoes for infection being selected Scotch seed.

The presumably infective insects for the experiments were raised as follows:

Six mosaic plants were grown in 12-inch pots, each plant confined under a tall glass cylinder fitted with a gauze top. On these six plants the six species of insects used were fed. In every case ample time was allowed for a given species of insect to become infected; in most cases, and especially with aphides, the insects were bred upon the infected potato plant. The capsid bugs were captured in the first and second larval instars and allowed to come to maturity under the glass cylinder upon the mosaic plant.

(*a*) *Inoculation experiments with insects upon the sprouts of tubers.* These inoculations were entirely negative in their results and as such will be only briefly outlined. A number of half-tubers of President (other halves as control), were placed in glass vessels with canvas tops. On the sprouts of the experimental halves the infected insects under trial were placed, the number of insects introduced on each half-tuber

varying from six to twelve. It was found that this method of inoculation was extremely unsatisfactory for all the insects except the aphides. The leaf-hoppers and white-fly died without feeding upon the sprouts, and the capsid bugs were not observed to puncture the sprouts.

The insects were placed upon the tubers on June 4th; they were removed on June 16th and the tubers then planted.

All the plants resulting from both experiment and control were healthy with the exception of (a) and (a_1) (*Myzus persicae*) where leaf-roll disease developed in both experiment and control.

(b) *Inoculation experiments with insects upon the haulm.* These experiments were carried out upon two plots of land at the University experimental grounds, Fallowfield, Manchester. On Plot 1, the larger of the two, twelve selected tubers of President were used. Each of these was divided into two, and each half was grown under an insect-proof cage, thus giving twenty-four cages on this plot. The half-tubers were planted and earthed up, and the cage placed in position at the time of planting to avoid any accidental insect contamination. Prior to planting, the tubers had been kept in an insect-proof receptacle and each tuber was examined before being planted for any signs of sprout-infesting aphides or other insects.

Plot 2 was treated similarly, using Great Scot in the place of President. In this case only twelve cages giving six experimental half-tubers and six controls were used, instead of twenty-four as with President.

A photograph of one of these experimental plots is shown in Plate VIII, fig. 1, the frames being lettered in the case of President, as follows:

President.

Aphididae.	*Myzus persicae*	G, G_1
	,, ...	H, H_1
	M. solanifolii	I, I_1
	,, ...	J, J_1
	M. circumflexus	K, K_1
	,, ...	L, L_1
Aleurodidae.	*A. vaporariorum*	M, M_1
	,, ...	N, N_1
Capsidae.	*Calocoris bipunctatus*	O, O_1
	Lygus pabulinus	P, P_1
Typhlocybidae.	*Eupteryx auratus*	Q, Q_1
	Zygina pallidifrons	R, R_1

The second symbol, G_1, H_1 etc., indicates the control in each case. It will thus be seen that two tests were made with each of the three species of aphis and with the white-fly, but only one with each of the two species of capsid bug and leaf-hopper. With Great Scot the twelve cages were numbered 1 to 6, and 1_a to 6_a, the latter being the controls, thus giving one test to each species of insect.

Description of transmission experiments with variety President.
Plot I.

The twenty-four half-tubers were planted and the cages placed in position on May 14th, 1925; and the following details are given concerning each experiment:

Myzus persicae (insects from sprouting potato tubers). Twenty-four wingless females were placed on each of the two experimental plants on June 10th, the plants being then 6 to 8 in. high, healthy and free from insects. Four weeks later the two experimental plants were cleared of aphis by spraying. No accidental infection by other insects took place in either experiments or controls, all four plants were inspected at intervals and remained healthy to the end of the season when the plants were dug up and the tubers collected for growing the following year. *G* gave six tubers, G_1 gave four tubers, *H* gave six tubers, H_1 gave two tubers.

Macrosiphum (*solanifolii*) *gei*. The insects originally came from a rose. The dates, numbers of insects and general procedure, were the same as in the preceding case. The plants remained apparently healthy throughout the season and no accidental contamination of either experimental or control plants took place. On harvesting, *I* produced eight tubers, I_1 produced three tubers, *J* produced five tubers and J_1 four tubers.

Myzus circumflexus (insects from chrysanthemum). Details of this experiment are as with the two other aphis species.

In view, however, of the results obtained in 1926, after planting out the resulting tubers, the observations on these inoculations during 1925 are given in full, as follows:

K. June 3rd. Plant 6 in. high apparently healthy, no insects present.
,, 10th. *M. circumflexus* introduced. Plant apparently healthy.
,, 17th. Traces of mottling visible on the young leaves.
,, 24th. July 2nd and July 6th. Mottling still present.
July 7th. Plant sprayed.
,, 13th. Aphides dead, mottling and puckering present, suggestive of mild mosaic.

K_1. June 19th. Plant apparently healthy, no insects present.
" 24th. Slight signs of mottling.
July 2nd. Mottling still present.
" 6th. Mottling less distinct.
" 13th. Apparently healthy, no insects present.

L. June 3rd. Plant 6 in. high, no insects present, apparently healthy.
" 10th. *M. circumflexus* introduced.
" 17th. Slight mottling visible on young leaves.
" 24th. Mottling still present.
July 2nd. Mottling more pronounced.
" 6th. Mottling and puckering developing.
" 7th. Plant sprayed.
" 13th. Aphides dead, mottling still present.

L_1. June 19th. Plant healthy, no insects present.
" 24th. Some signs of mottling.
July 2nd. Some symptoms of mosaic.
" 6th. Symptoms increased.
" 13th. Symptoms still persisting, plant free of insects.

On harvesting, *K* gave three tubers, K_1 gave five tubers, *L* gave seven tubers (very small), L_1 gave three medium-sized tubers.

Asterochiton vaporariorum, white-fly.

Twenty-four individuals of the greenhouse white-fly were placed upon each of the two plants *M* and *N* on June 10th, the plants being then about 6 in. high, healthy and free from insects. The white-fly continued to feed on these potato plants until July 13th when the plants were sprayed. Both experimental and control plants remained apparently healthy throughout the summer, and on harvesting gave the following numbers of tubers: *M* gave four tubers, M_1 gave two tubers, *N* gave six tubers, N_1 gave three tubers.

Calocoris bipunctatus, *Lygus pabulinus*, Capsid bugs.

On June 29th, twelve individuals of the two species of capsid bug were placed upon the plants *O* and *P*. *C. bipunctatus* on *O*, and *L. pabulinus* on *P*. On July 13th the plants were examined, the capsid bugs were still living and the plants showed a certain amount of damage from the feeding of the bugs, but were otherwise healthy.

Both plants and their controls remained healthy throughout the summer. On harvesting, *O* gave three tubers, O_1 gave two tubers, *P* gave three tubers, P_1 gave five tubers.

Eupteryx auratus, *Zygina pallidifrons*, Leaf-hoppers.

Twelve individuals of *E. auratus* and twelve of *Z. pallidifrons* were placed on June 16th on the two plants *Q* and *R* respectively. The plants were inspected at weekly intervals till July 13th when they were sprayed, leaf-hoppers were still present on that date, and in the case of *Q*, the plant though badly marked by the leaf-hoppers appeared otherwise quite healthy. On June 22nd both *R* and R_1 showed unmistakable signs of mosaic infection, these two plants were therefore discarded and this experiment repeated. New half-tubers were planted on June 22nd and the insects placed on the plants on July 10th. Both *R* and *Q* and their controls remained apparently healthy except for the puncture marks of the leaf-hoppers. On harvesting, *Q* gave four tubers, Q_1 gave three tubers, *R* gave four tubers, R_1 gave four tubers (large).

Plot II.

In this plot as already described twelve cages only were used, giving six experimental frames and six controls, the variety Great Scot was substituted for President. The same insects were used as in the foregoing, there being, however, only one inoculation test with each species of insect, viz. one capsid (*C. bipunctatus*) and one leaf-hopper (*Z. pallidifrons*) being used.

The results of this experimental plot were entirely negative, both inoculated and control plants remaining healthy throughout the summer. On removing the cages and digging the plants at the end of September, it was found that no tubers had been formed; further observations were therefore impossible.

The tubers resulting from the plants on Plot I, variety President, were all small in size, although sufficient to carry on the plants the following year. A point of some interest arises from a comparison of the tubers produced by the inoculated plants of President with the uninoculated control plants, namely that the progeny of the controls was always superior in size, though not necessarily in number, to the progeny of the experimental plants. This the writer puts down to the deleterious effect of the feeding of the insects introduced under the frames. Such reduction in the size of the tuber was especially marked in plants *O*, *P*, *Q* and *R*, where the insects used, capsid bugs and leaf-hoppers, are particularly injurious to plant foliage.

The tubers were carefully collected, each being provided with its identification mark in Indian ink and stored in silver sand. The progeny of each plant was kept in a separate receptacle.

Later, the silver sand was discarded and the tubers were stored in canvas-covered dishes.

Results obtained in 1926 *from the progeny of the plants inoculated under cages in* 1925.

The tubers variety President from Plot I were planted in 1926 in a compost of leaf mould and soil contained in 10-inch pots. The pots were placed in a cold greenhouse which was free from aphis, having had no plants in it during the winter. Each pot was labelled and an additional wooden label was fixed in the soil. The following results were obtained:

APHIDIDAE.

Myzus persicae.

G. Six tubers, six plants, all mosaic.
G_1. Four tubers, four plants, all healthy.
H. Six tubers, six plants, all mosaic.
H_1. Two tubers, two plants, both healthy.

Macrosiphum gei (= *solanifolii*).

I. Eight tubers, eight plants, four healthy and four mosaic.
I_1. Three tubers, three plants, all healthy.
J. Five tubers, five plants, two mosaic, three healthy.
J_1. Four tubers, four plants, all healthy.

Myzus circumflexus.

K. Three tubers, three plants, all mosaic.
K_1. Five tubers, five plants, one mosaic, four healthy.
L. Seven tubers, seven plants, all mosaic.
L_1. Three tubers, three plants, all mosaic.

ALEURODES.

Asterochiton vaporariorum (white-fly).

M. Four tubers, four plants, two mosaic, two healthy.
M_1. Two tubers, two plants. Both healthy.
N. Six tubers, six plants, all healthy.
N_1. Three tubers, three plants, all healthy.

CAPSIDAE, Capsid Bugs.

Calocoris bipunctatus.

O. Three tubers, three plants, all healthy.
O_1. Two tubers, two plants, both healthy.

Lygus pabulinus.

P. Three tubers, three plants, all healthy.
P_1. Five tubers, five plants, all healthy.

Typhlocybidae, Leaf-hoppers.

Eupteryx auratus.

Q. Four tubers, four plants, one mosaic, three healthy.
Q_1. Three tubers, three plants, all healthy.

Zygina pallidifrons.

R. Four tubers, four plants, one mosaic, three healthy.
R_1. Four tubers, four plants, all healthy.

In Plates VIII–X are shewn photographs of some of the successful inoculations with their controls.

4. Discussion of Results of 1925 inoculations with insects.

Before considering the results of these inoculation trials, it will be well to review shortly the conclusions arrived at by other workers in this field. As regards Aphides, Murphy is of the opinion that several species are transmitters of this disease, but he finds that under apparently similar conditions inoculation is often not successful. In a recent paper(5) he says: "We have at different times successfully conveyed mosaic and leaf-roll infections from tuber to tuber by means of aphides on the sprouts, but on many occasions the results have been mainly or entirely negative. Thus, the percentage of successful infections varied from 100 to 0, and in spite of much work on the subject the factors governing this variation remain undetermined. Three different aphides have been used at various times, these being *Myzus persicae, Macrosiphum gei* (= *solanifolii*) and *Myzus pseudosolani.* All have carried infection at times so that the conflicting results do not depend on the sort of aphis used, neither do they appear to be determined by the number of aphides used, the length of their stay on the source of infection or on the plant being infected; by the medium by which the tubers are sprouted, whether air or soil, by the presence or absence of light nor by the temperature."

These experiments were attempts at transmission from the sprouts of infected tubers to sprouts of healthy tubers, and not as was the case in the writer's experiments from infected haulm to healthy haulm. Schultz and Folsom(8) report successful inoculation by means of three species of aphides, *Myzus persicae*, *Macrosiphum gei*, and *Aphis*

abbreviata Patch. These authors find that aphides produce the symptoms of virus disease in the same year when caged upon the haulms, though apparently they also find difficulty with aphis inoculations, as they state ((8) p. 526) that: "Aphides sometimes do not transmit disease under conditions that apparently are the same as those giving positive results."

In an earlier paper(6) Schultz and Folsom find that plants treated with virulent aphides may appear healthy but produce progeny that are all affected with mosaic, and again in the same Journal(7) they state that: "*Macrosiphum gei* transmitted mosaic to a number of plants with symptoms only in the progeny." These findings are of interest in view of the writer's results with the same species of aphis.

As regards other insect carriers of potato virus opinion appears to be divided. Murphy(4) seems to be of the opinion that various potato insects other than aphides are carriers of leaf-roll, though not necessarily of mosaic. He gives the following insects as carriers: the capsid bug *Calocoris bipunctatus*, and the Jassid *Typhlocyba ulmi*. To the writer's knowledge the last-named Jassid does not attack potatoes, so possibly the species referred to was *Eupteryx auratus*, which somewhat resembles it and is a common potato insect. Murphy gives his conclusions as follows: "While the above results can show hardly more than a suspicion that the potato flea-beetle can act as a carrier of leaf-roll there is little room for doubt that both capsid bugs and jassids act as efficient transmitters of leaf-roll. This is important as showing that there is no exclusive specific relationship between aphides and the actual cause of leaf-roll which is presumably an organism."

In 1920 Schultz and Folsom(6) stated that: "As yet no other insect (than aphides) is known to carry mosaic of potato." Since then, however, this opinion may have been modified.

There seems little doubt that insects other than aphides act as carriers of virus disease in many plants of different orders. The work of Kunkel(2) and Hartzell shows that the leaf-hopper *Cicadula sexnotata* is a carrier of the virus disease known as "Aster Yellows." Storey(10) has also shown that a species of leaf-hopper transmits a virus disease of maize known as "Streak." In addition various leaf-eating beetles are thought to be carriers of mosaic of other plants.

Turning now to the results of the present preliminary investigations, some definite evidence of successful transmission of potato mosaic by means of certain insects appears to emerge. It will be seen that with most of the insects one or other of the two inoculations with each insect

is apparently successful. The chief point of difference seems to lie in the *amount* of infection produced by the various insects. Thus in the case of the aphis *Myzus persicae* the six tubers harvested from *G* and the six from *H* gave mosaic infected plants in every case. On the other hand, in *Q* and *R* the inoculations with the two leaf-hoppers produced only one infected plant out of four, the white-fly gave two out of four in one case and none at all in the other. The capsid bugs failed to transmit the disease in both cases. The insects thus appear to vary greatly in the degree of their efficiency as disseminators of virus disease. This may be due either to the method of feeding of the insects or to some requirement of the virus. It may also be suggested that plants *Q* and *R* which only gave one infected plant out of four did not receive the same amount of virus as did plants *G* and *H*, or else successful inoculation was only achieved in one or two stems of the plant. Schultz and Folsom (6) find that a plant with three stalks healthy and four mosaic may produce three mosaic tubers and two healthy ones. The number of insects used in each experiment may influence the infection in so far as even distribution over the plant is concerned. In the case of the aphis *Myzus persicae*, out of twelve tubers from the 1925 inoculations, twelve mosaic plants were produced in 1926, while the six control plants remained healthy.

With the second aphis, *Macrosiphum gei*, six mosaic plants were produced out of the total of thirteen tubers from the two inoculated plants, the seven control plants remaining healthy.

It is unfortunate that the trials with the third species of aphis *Myzus circumflexus* for 1925 must be disregarded owing to the failure of the controls to remain healthy.

It is manifestly unwise to make definite statements as to the infective power of these insects at this stage in the investigations, nevertheless it is possible to draw some conclusions. It seems fairly clear that *under certain conditions*, the aphides *Myzus persicae* and *Macrosiphum gei* can act as efficient transmitters of potato mosaic disease. In the present state of our knowledge of this subject it is not possible to say what these conditions may be. Unlike Schultz and Folsom the writer has so far been unable to produce symptoms of mosaic disease in a potato plant in the same year as that in which the inoculations were performed by means of aphides placed upon the haulm. The disease has only shown itself in the plants produced by the progeny of the experimental plant in the following year. In the case of inoculation with aphides, however, upon sprouting tubers, it appears possible to

produce current season symptoms. Atanasoff(1), commenting upon the difficulty and uncertainty of aphid inoculation of the haulm, states that most of the difficulties would disappear if the inoculations were made with the sprouting tuber and the experiment thus confined to one season. Although this is probably true of some aphis inoculations, it does not hold good for transmission experiments with other potato insects, such as capsid bugs, leaf-hoppers and flea-beetles, as these will not feed upon the sprouts of tubers or, in the case of the capsids, can only with great difficulty be induced to do so. For these insects the only method of sprout inoculation would seem to be the removal of the salivary glands and insertion of them into the sprout. This is being carried out in the present season (1926).

Apart from this there seems no alternative to the method of two-season haulm infection, and there is no reason why this should not be reliable if proper care be taken in carrying out the experiments.

In considering the inoculations with white-fly (*Asterochiton*) it is interesting to find infection in one case. Although more cannot be said at this early stage of the work, it seems quite probable that the white-fly is a potential carrier of virus disease, more especially as it is an insect which, like the aphides, habitually taps the phloem in its feeding. Both species of capsid bug failed to transmit the disease, thus in some measure bearing out the suggestion of the writer(9) that insects such as these which possess a salivary secretion violently toxic to the plant host are unlikely to be transmitting agents.

With the two leaf-hoppers one plant only with each species became infected. This very slight infection may be due either to the toxic effect of the saliva upon the plant, or to the methods of feeding of these insects which only occasionally tap the phloem. Leaf-hoppers, especially *Eupteryx auratus*, are sometimes severe pests of the potato plant as in the present summer (1926), and it is important that their connection, if any, with the spread of virus diseases of the potato should be established.

Some possibilities in these insect inoculations of the potato plant must be considered. Firstly the effect of the canvas cages upon the normal growth of the plant, and secondly their effect on the symptoms of mosaic disease. In the 1925 experiments it was found that the plants under the cages were drawn up and sappy, and had to be supported. In addition the tubers appeared to be reduced in size as compared with those of plants grown normally. Apart from this the plants grew strongly and were green, luxuriant and apparently healthy enough.

In regard to the second point it is difficult in the present state of our knowledge to say what the effect of the cages and the consequent alteration of light and temperature would be on the development of virus disease. Atanasoff (1) says on this point: "The potato can grow and develop a normal and very luxuriant growth at comparatively low temperatures if only sufficient sunlight is present. Most of the virus diseases of this plant, on the other hand, require much higher temperature for their development, so that their development can be not only retarded but even completely suppressed by low temperatures which are high enough to permit a normal development of the plant."

Atanasoff goes on to say that it is thus possible for a positively infected tuber to produce under low temperature conditions an apparently healthy plant, thus giving a partial or complete suppression of the disease.

Murphy (3) on the other hand, would appear to hold the opposite view, he says: "Field experiments on mosaic were rendered nugatory so far as the greater part of 1921 was concerned, by the very dry and hot weather which had the effect of suppressing the symptoms partially or almost entirely. It was already known from observation and experiment in Canada (as well as in U.S.A.) that this might happen in certain warm and dry localities and seasons. Emphasis was there laid on the temperature, but the appearance of some mosaic symptoms for the first time in plants presumed infected from the beginning, following the first good rain towards the end of July, would indicate that moisture may also be concerned. It was clearly shown in some of the Canadian experiments referred to, that though the symptoms were suppressed in the case of diseased plants in certain places, with the result that their yield was hardly inferior to that of neighbouring healthy plants, yet the disease was still present and transmissible. It reappeared in the diseased seed in the subsequent year, under conditions more favourable to the disease in at least as intensified a form as before its suppression."

Murphy also says: "27° C. is the optimum for the growth of the potato and this exceeds that necessary for the development of the hypothetical mosaic organism. The latter may be concluded from the fact that the disease is most flourishing under cooler conditions."

In regard to the effect of light upon the development of virus disease Schultz and Folsom (7) state: "Shading tended to increase mosaic mottling and decrease leaf-rolling."

In 1925 when the writer's experiments were carried out the summer was exceptionally hot and dry. The plants, however, were kept well

watered by flooding the bases of the insect-proof frames. It may be noted that in the case of L and L_1 and the first R and R_1, where the tubers were already affected, the mosaic disease developed normally, the rest of the plants remaining green and apparently healthy till the end of the season. From this it would appear that the presence of the canvas cages does not to any great extent affect the development of the disease symptoms.

Summary.

1. An account is given of some preliminary experiments with insects as transmitters of potato mosaic disease. The various types of insect-proof cage which have been used in these experiments are described.

2. The following insects were used in the inoculations:

Aphididae.

Myzus persicae.
Macrosiphum gei (= *solanifolii*).
Myzus circumflexus.

Aleurodes, White-fly.

Asterochiton vaporariorum.

Capsidae.

Calocoris bipunctatus.
Lygus pabulinus.

Typhlocybidae, Leaf-hoppers.

Zygina pallidifrons.
Eupteryx auratus.

3. The aim of the experiments is definitely to identify what insects of those normally attacking the potato are disseminators of virus disease.

4. Infected insects were placed both upon the sprouts of tubers and upon the haulm; the latter is the only satisfactory method for insects other than aphides.

5. Successful transmission of mosaic disease was obtained in 1925 by means of the aphides *Myzus persicae* and *Macrosiphum gei*. Some evidence of infection by means of *Asterochiton vaporariorum* (greenhouse white-fly) and the leaf-hoppers *Zygina pallidifrons* and *Eupteryx auratus* was also obtained; further work with these insects is required before definite conclusions are drawn. The capsid bugs *Lygus pabulinus* and *Calocoris bipunctatus* failed entirely to transmit the disease.

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EXPLANATION OF PLATES VIII—X

PLATE VIII.

Fig. 1. View of one of the experimental plots showing cages in position. Each cage is held firmly in place by an external wooden frame.

Fig. 2. Successful transmission of mosaic disease by means of the aphis *Macrosiphum (solanifolii) gei.* This plant commenced with mild mosaic, but at the time the photograph was taken late in the season, it was in a curly dwarf condition.

Fig. 2 a. Healthy control plant.

PLATE IX.

Fig. 3. Successful transmission of mosaic by means of the aphis *Myzus persicae.*

Fig. 3 a. Healthy control plant.

PLATE X.

Fig. 4. Successful transmission of mosaic by means of the leaf-hopper *Zygina pallidifrons.*

Fig. 4 a Healthy control plant.

Fig. 5. Leaves from plant in Fig. 4 enlarged to show mosaic symptoms. All the plants are variety "President."

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THE LIFE HISTORY AND BIONOMICS OF A BRITISH PHYTOPHAGOUS CHALCIDOID OF THE GENUS *HARMOLITA* (*ISOSOMA*)

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(With 12 Text-figures.)

CONTENTS.

1. Introduction.

Although many economically important species of the genus *Harmolita* have been studied in different parts of the world, notably in the United States of America and in Russia, nothing is known of the biology of this genus in Great Britain. It was therefore considered desirable to investigate the life history and biology of British species of *Harmolita*.

Several species of *Harmolita* which are gall formers on the common couch grass (*Triticum repens*) proved to have a very wide prevalence in this country and consequently formed a convenient source of material for this investigation.

The life history of one of these species attacking *Triticum repens*, namely *Harmolita graminicola* (Gir) has been described with full details; one other undetermined species whose larva is also gallicolous on couch grass has been figured. Its life history approximates very closely to that of *Harmolita graminicola*.

The latter species was successfully induced to breed in captivity on *Triticum repens* but refused to breed on wheat or any other available representative of the *Triticum* genus.

It is quite possible however that closely allied species may be gall formers on cultivated wheat although I have not yet succeeded in finding the wheat plant infested with species of *Harmolita* in this country.

So far, also, none of the British species of *Harmolita* examined exhibited the interesting phenomenon of complete change of diet reported by several investigators concerning some foreign species of this genus. Thus Rimsky-Korsakov (5), whilst working on the Russian *Harmolitae* attacking cereals found that one of his species named *Harmolita inquilinum*, oviposits in the galls of another species of the same genus (*H. rossicum*), and that its larva feeds first on the larva of the gall maker and then finishes its development phytophagically. Phillips (4) reports that the larva of *Eurytoma pater*, a genus closely allied to *Harmolita*, is a true parasite in its early stages of the larva of an *Harmolita*, and that it consumes the host larva before completing one-third of its own growth and then finishes its development on plant tissue. Nielsen observed a similar phenomenon in another Eurytomid.

These facts are considered by Gahan (1) to suggest that phytophagy in Chalcidoidea is a recent specialisation forced upon the chalcid parasite by a premature exhaustion of the natural food supply. This research was commenced in the Zoological department of Manchester University, and completed in the Zoological section of the department of Agriculture in the Leeds University. I must here express my obligation to Professor S. J. Hickson, F.R.S., of Manchester, and Professor R. S. Seton, and Mr T. H. Taylor of Leeds, for facilities to carry out this work. My thanks are also due to Miss E. M. Wright and to Miss M. Jepson for drawing some of the text-figures.

2. Methods of Investigation.

The fact that the whole of the post-embryonic developmental period of species of *Harmolita* is passed enclosed within galls is productive of certain difficulties in the investigation of individual life cycles. The opening of a gall containing a larva in one of its trophic stages is tantamount to the latter's destruction, even though the minute and delicate creature be uninjured in the process. This is due to the drying up of the tissues of the gall which become no longer acceptable as food to the larva.

Hence it is impracticable to follow any one specimen through the

various stages of its life cycle. When, however, a larva has become full fed and commenced to hibernate it may be removed from the gall as the latter is then only required for protective purposes. It was found that when hibernating larvae were removed from their galls, and enclosed under an inverted watch-glass upon a sheet of filter-paper they would develop normally if kept in the dark and left at the prevailing seasonal temperatures.

Galls collected in October, when the great majority of the larvae had commenced to hibernate and afterwards maintained in the laboratory at a fairly uniform temperature of about 60° F. produced a remarkable acceleration in their rate of development in response to the stimulus of higher temperatures. Emergence of the adults could be made to take place as early in the year as February, which was about four months before the normal time.

Rimsky-Korsakov (5), observed a somewhat similar phenomenon when studying Russian species of *Harmolita*. Whilst studying the stages subsequent to hibernation, this method of removal from the galls was found superior to that of continually taking out and replacing larvae in the galls, for however careful the operator may be, larvae are liable to be killed or so injured that a retardation or distortion of growth takes place.

In order to study the early stages of the life cycle *Harmolita graminicola* was successfully bred in captivity. The procedure adopted was as follows: "Roots" of couch grass (*Triticum repens*) bearing young shoots were transplanted into fairly large plant pots with a diameter of about a foot and a height of about a foot and a half. Ten of these pots were employed and each contained numerous young shoots of *Triticum repens*. Several precautions are necessary for success. First, if the inflorescence has burst through the sheathing leaves no attempt at oviposition will be made on the grass culm. Second, transplanting should be undertaken well before the time of emergence in order to give the plant time to get over its ill effects and to ensure that eggs have not already been deposited in its culms.

The plants were then enclosed in glass cylinders whose open tops had been covered with muslin. The cylinders have a height of about two feet and were of such a diameter as to fit easily on the tops of the plant pots. The pots were kept during the breeding experiment in an open insectary at the Manchester University Experimental Grounds at Fallowfield. The insectary has a corrugated iron roof, but the sides are only protected by a large mesh wire netting thus ensuring natural conditions of light and temperature.

Galls were collected as near to the time of emergence as possible and the adults allowed to emerge under glass cylinders similar to those enclosing the potted grass plants. After emergence the insects were left undisturbed for three days in order to ensure maturation and fertilisation (if the latter is really necessary) of the eggs. At the end of this period the cylinders enclosing the plants were removed and replaced by cylinders containing the adult insects.

Oviposition then took place. The insects were allowed to remain in contact with the plants for 24 hours; this period of time was found sufficiently long to ensure oviposition.

3. Time of Emergence and Longevity of Imagines.

The period of normal emergence of the adults extends from about June 24th to July 7th. The imago escapes by eating out a circular hole in the wall of the gall with its mandibles. The aperture through which it emerges is not confined to any particular spot on the wall of the gall. The first act of an imago after emergence is to remove particles of plant debris from off its body and limbs. This done the insect proceeds to rear itself up on the posterior apex of its abdomen and to work off, by means of its prothoracic legs, the old pupal envelope still encasing each antenna. At least an hour elapses before the newly emerged insect shows a desire to use its wings. The life of the imagines may be of considerable length for they have been kept alive in captivity without access to food or water for 14 days. Those which oviposited in the breeding cages did not die soon afterwards but appeared to live about as long as the males in whose company they were kept. Probably a month represents the normal duration of life of the adult *Harmolita*. They were observed when at liberty feeding on the pollen of various grasses which appears to constitute their chief source of food.

4. Parthenogenesis.

The evidence is strongly against the occurrence of parthenogenesis in *Harmolita graminicola*, although there is still some doubt concerning the matter. The sexes occur roughly in about equal numbers during the only generation of the year. Several experiments were conducted in an endeavour to determine with certainty whether fertilisation was necessary. A number of galls were opened shortly before the time of emergence to ascertain the sex of the occupants. A number of female pupae were collected in this manner, placed in breeding cages and allowed to mature. The resulting female flies without being afforded an

opportunity of fertilisation were allowed to come into contact with *Triticum repens*. Extensive oviposition took place but no galls subsequently resulted. These results, however, may still be regarded as inconclusive, and a repetition would be desirable especially in view of the fact that the breeding of these chalcid flies is a somewhat delicate operation, and some other factor distinct from non-fertilisation may have intervened to prevent the development of the eggs.

5. Oviposition.

The opportunities of watching the act of oviposition were many and the method of egg laying appeared to be as follows:

The fly crawls along the grass culm tapping it at frequent intervals with its antennae. The latter organs appear to be acting in an investigative capacity. When an apparently suitable spot is reached the front end of the body is raised and the hinder end lowered until the first two pairs of limbs hang free and the posterior tip of the abdomen is in contact with the surface of the grass culm, the insect being pivoted on its metathoracic legs. The two palp-like continuations of the inner plates can be seen adjusting the tip of the ovipositor sheath to the exact spot where the puncture is to be made. The ovipositor sheath with its contained stylets are then driven into the plant tissue by a backwardly directed sliding movement of the abdomen. The sheath by this movement is drawn away from its customary position along the ventral surface of the abdomen and makes an angle of from 45° to 90° with the ventral surface of the abdomen. The ovipositor sheath is now driven its full length into the plant by the approximation of the ventral surface of the abdomen to the leaf sheath.

The time during which the ovipositor remains inserted varies. The writer has seen its retention in the plant for the period of a minute but more frequently it has been withdrawn after 10 seconds. During the time of insertion the ovipositor is partially withdrawn and pressed in again several times. Only one egg is laid in each grass culm, and this was shown to be invariably the case by a series of dissections of grass culms immediately after oviposition in them.

The egg is always deposited in the centre of the stalk about 2·5 mm. below the tip of the rudimentary inflorescence. The egg consists of an elongated oval body with a fairly long pedicel at one pole and a very short rudimentary one at the other. The chorion is smooth and transparent and the developing embryo can be seen quite well within, under suitable optical conditions. The length of the body of the egg excluding

Fig. 1.

Fig. 2.

Fig. 2A.

SMITH.—Observations on the Insect Carriers of Mosaic Disease of the Potato (pp. 113–131).

Fig. 3.

Fig. 3A.

SMITH.—Observations on the Insect Carriers of Mosaic Disease of the Potato (pp. 113–131).

Fig. 4.

Fig. 4A.

Fig. 5.

the pedicel is generally just a little short of 0·4 mm. The body of the egg passes anteriorly down the ovipositor, and its passage is thus facilitated, as part of the contents of the body appears to pass into the pedicel during the passage down the ovipositor. No part of the egg protrudes beyond the epidermis after oviposition has taken place.

6. Period of Incubation.

After oviposition had taken place in the manner described above endeavours were made to ascertain the length of the period of incubation. Dissection of a culm containing an egg was made daily from each of five pots used for this experiment for a period dating from oviposition to the day a larva was found. If it happened that a grass culm did not contain an egg another one was taken from the same pot until one containing an egg was discovered. On the day a larva was first found all the remaining culms in that particular pot were dissected.

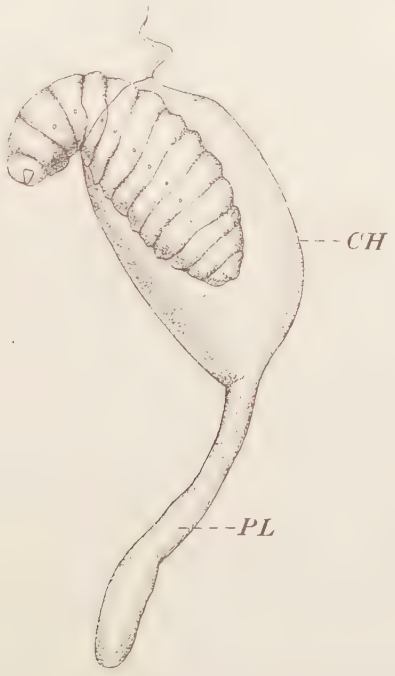

Fig. 1. The eclosion of the larva from the egg × 120 diams. Drawn from a Canada balsam mount stained with Delafield's Haematoxylin. The larva has shrunk proportionately more than the egg membrane.

CH, Chorion of egg. *PL*, Pedicel of egg.

The period of incubation was found to be a comparatively long one as the following table shows:

Pot number	Date of oviposition	Grass culms dissected	Eggs dissected out	Date larva first found
1	June 30th	37	27	July 24th
2	July 1st	30	23	—
3	,, 1st	42	15	—
4	,, 1st	35	27	July 23rd
5	,, 1st	44	29	,, 27th

The above table shows pretty conclusively that the period of incubation lies between three and four weeks for *Harmolita graminicola*. In Pot 1, as shown by the table, the first larva was dissected out on July 24th and from the remaining culms on the same day four eggs and five larvae were obtained. From Pots 2 and 3 all the eggs were dissected out before the period of incubation was over, consequently no larvae

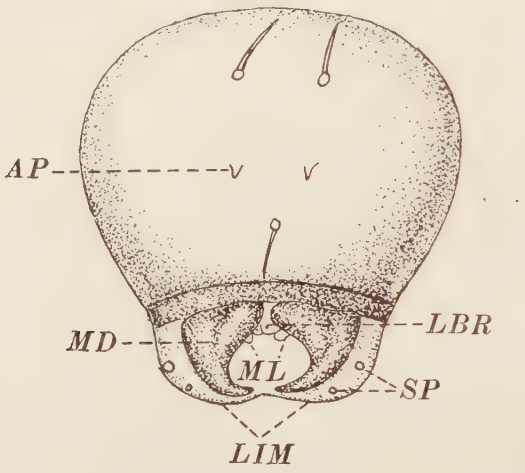

Fig. 2. The frontal view of a cephalic segment of a newly hatched larva about 0·5 mm. long, showing the complete absence of sub-apical teeth on the mandibles × 300 diams. Drawn from a potashed mount stained with 2 per cent. acid fuchsin.

AP, Antennal papilla. *LBR*, Bi-lobed labrum bearing two sense spots. *LIM*, Bi-lobed labrum. *MD*, A mandible. *ML*, Maxillary lobes. *SP*, Sense spots of labium.

were obtained from these pots. In Pot 4 the first larva was found on July 23rd, and from the remaining culms dissected out on the same day five eggs and four larvae were obtained.

In Pot 5 the first larva was found on July 27th, and from the remaining culms three eggs and seven larvae were obtained. The eggs examined in the fourth week after oviposition showed unmistakable

signs of the nearness of hatching and the rapidly developing larva could be seen within. The body of the egg increases in size as the time of hatching approaches and a tension is set up in the egg membranes. The larva escapes from the egg at the pole opposite to that at which the long pedicel is situated. The larva does not possess any specialised

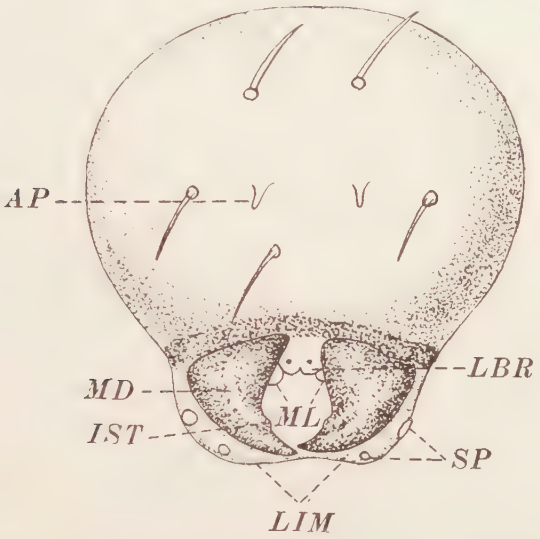

Fig. 3. The frontal view of a cephalic segment of a larva of the second instar, measuring about 1·3 mm. long, showing the incipient sub-apical tooth of the mandibles × 200 diams.

IST, Incipient sub-apical tooth of a mandible. Other lettering as in Fig. 2. Drawn from a potashed mount stained with 2 per cent. acid fuchsin.

hatching apparatus and appears to rupture the already tense chorion by means of its mandibles assisted no doubt by movements of the body.

Fig. 2 shows a larva in the act of escaping from the transparent egg membranes and the part of the larva still within the egg could be clearly seen.

7. The Larva of the First Instar.

The newly hatched larva varies in length from 0·4 to 0·5 mm. and is usually found adhering to the empty egg-chorion. The segmentation is clearly defined, and consists of a head and 13 body segments. The cephalic segment is smaller in proportion to the segments immediately behind it than in larvae of later instars, and the antennae papillae seem to be more conspicuous.

The hinder part of the body tapers more gradually and is more pointed than in older larvae. Unlike many parasitic chalcid larvae the alimentary canal is continuous from mouth to anus from the time of hatching as expulsion of liquid excrement from the anus was observed almost immediately after hatching. It is easy to ascertain whether a larva has begun to feed, because if food has been taken the mesenteron

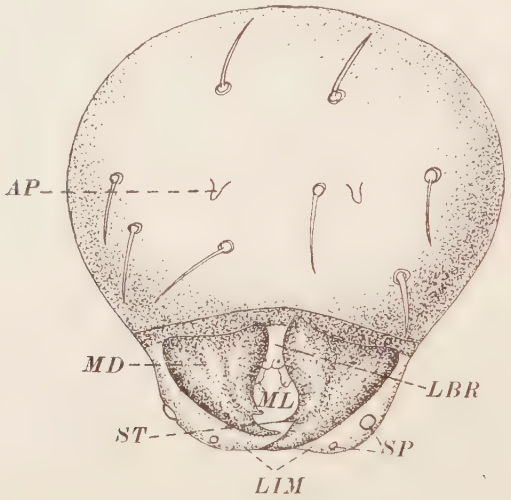

Fig. 4. A frontal view of a cephalic segment of a larva of third instar about 2·1 mm. long, showing the development which has taken place in the sub-apical tooth of the mandible × 150 diams. Drawn from a potashed mount stained with 2 per cent. acid fuchsin.

ST, Sub-apical tooth of the mandible. Other lettering as in Fig. 2.

will exhibit a distinctly green colour which can be observed very clearly through the almost transparent body. Little lobulated masses, slightly more translucent than the general content of the body can be discerned here and there beneath the transparent integument. These bodies form the basis of the large mass of fat body which subsequently develops and finally renders the larvae of the later stages completely opaque.

The respiratory system is the same fundamentally as in larvae of later stages; it consists of a pair of longitudinal tracheal trunks united transversely, both anteriorly and posteriorly by commissures.

The longitudinal trunks give off at segmental intervals lateral branches to the spiracles. The spiracles can be recognised as nine in number on each side, a pair being situated on each segment from the second to the tenth inclusive. The respiratory system is peripneustic from birth with closed spiracles on the wing-bearing segments. The air

tubes show up clearly in freshly dissected larvae of this instar owing to the relative absence of fat body. By suitable staining various internal structures such as the salivary glands and central nerve cord can be observed. Faint indications of the cutaneous muscular system can also be seen. The feature distinguishing the first instar larva is found in the mouthparts.

The mandibles (Fig. 2, *MD*) do not each possess a sub-apical tooth which is present incipiently at least in all later instars (Fig. 3, *IST*, and Figs. 4 and 5, *ST*). There is a prominent slightly bi-lobed labrum

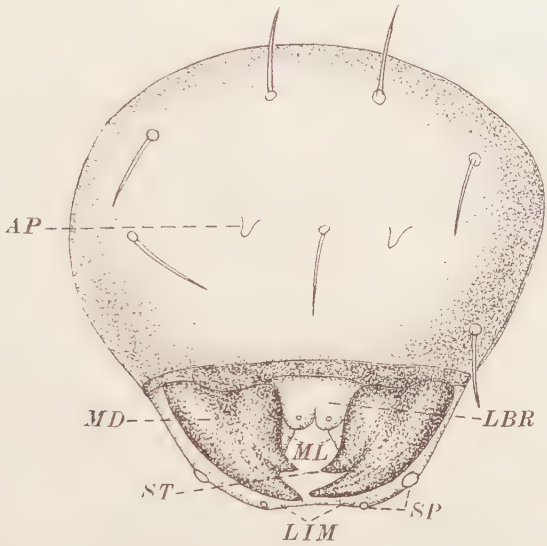

Fig. 5. A frontal view of a cephalic segment of a mature larva showing the fully developed sub-apical tooth of the mandible × 130 diams. Drawn from a potashed mount stained with 2 per cent. acid fuchsin.

ST, Sub-apical tooth of the mandible. Other lettering as in Fig. 2.

(Fig. 2, *LBR*) bearing a small sense spot on each lobe. A pair of maxillae are also represented by two lobes (Fig. 3, *ML*). The labium (Fig. 2, *LIM*) is very large and indented slightly in the median line to give it also a somewhat bi-lobed appearance. Each of these lobes bears two sense spots (Fig. 2, *SP*) one of which is relatively very large and presents an hemispherical face.

A larva after it has been feeding for a day or two may be found in a little cavity eaten out by itself just beneath the germ of the future inflorescence which is destined never to shoot the enveloping sheath and ripen into seed. Whilst growing to about 1 mm. in length a larva does

not produce any external indication of its presence within the stem. Subsequently an elongated swelling begins to form above the uppermost node of the stalk and when the larva is 2 mm. long it is quite apparent.

8. Instars Later than the First.

The first moult takes place when the larva is about one week old and measures about 1·3 to 1·5 mm. long. Apart from size and a slightly greater translucence of the body content the larva of the second instar

Fig. 6. A side view of a mature larva of *Harmolita graminicola* (Gir) × 50 diams.

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Fig. 7. A side view of a mature larva of an undetermined species of *Harmolita* × 50 diams.

differs from that of the first only in that the mandibles of the former exhibit indications of sub-apical teeth (Fig. 3, *IST*). The second stadium lasts about a fortnight when another ecdysis is effected, the length of the larva being then about 2·0 to 2·2 mm. In this, the third instar of

the larva, the sub-apical teeth of the mandibles (Fig. 4, *ST*) are much better developed. The third stadium also lasts about a fortnight and when the larva measures about 3 mm. long the cuticle is cast for the third time.

This is the last moult prior to the one at pupation. In the last instar the sub-apical teeth of the mandibles are fully developed (Fig. 5, *ST*). The body contents have become quite opaque owing to the increase in size, number, and density. In the month of October the great majority of the larvae reach the fully fed condition and they then enter into a very quiescent state. In this state of hibernation the fully fed larva passes the winter under the protection of the gall.

9. The Pro-nymph or Semi-pupal Stage.

Pupation begins to take place about the end of April but at least 90 per cent. pupate in the middle of May. As the time of pupation draws near the old larval skin begins to lift in preparation for the moult. About 48 hours before pupation takes place, the larval body assumes a permanent flexure at the region of the third and fourth body segment, and the sternal region of the fourth segment becomes almost obliterated. About this time a series of rhythmic risings and fallings of the larval cuticle can be detected. These are brought about by the circulation of the moulting fluid between the larval cuticle and the new pupal cuticle beneath. They are specially discernible in the lateral abdominal region in the neighbourhood of the spiracles where a ridge-like puckering is formed by repeated risings and fallings of the cuticle.

The moulting fluid after permeating between and aiding in the separation of the old larval cuticle, from the underlying coat, is gradually worked by a series of wavelike motions to the dorsal region of the thorax. The cephalic and thoracic segments become swollen and paler in colour owing to the pressure caused by the underlying accumulation of fluid. This is the true semi-pupal stage corresponding to that described in some other Hymenoptera (3), and lasts from 12 to 24 hours in this case. The moulting fluid first causes the swelling or "blister" on the whole of the dorsal thoracic region, but later the fluid is forced forward and heaped up over the dorsal prothoracic region and the tension in the cuticle at this spot becomes very great. Then by an increase in the flexure of the body at the region of the third and fourth segments already referred to and by vigorous wriggling in the abdominal region the breaking point in the swollen prothoracic region is reached. The rupture of the old larval integument which begins in the median line of the

Fig. 8.

Fig. 9.

Fig. 8. Female adult of *Bracon erythrostictus* (Lyle) parasitic on *Harmolita* in the larval stage × 50 diams.
Fig. 9. A parasitised larva of *Harmolita graminicola in situ* in the gall × 10 diams.

dorsal prothoracic region rapidly extends in a longitudinal direction over the whole length of the dorsal surface of the thorax and is followed by a liberation of the moulting fluid and the rapid emergence of the pupal head which lay in the prothoracic region of the larva.

The old larval integument with the mandibles attached is gradually worked towards the posterior region of the body by repeated movements of the abdomen and by occasional flexion of the entire body about the fourth segment. When isolated from the gall the act of slipping out of the larval cuticle takes from 40 minutes to an hour, and the process may take a considerably shorter time inside the gall. Finally the old larval skin is forced back to the posterior extremity of the abdomen where it remains attached almost throughout the pupal stage as a dried appendage attached to the posterior extremity of the abdomen.

10. The Pupal Stage.

Immediately after pupation has taken place the pupa presents a very pale yellow colour and no black markings are discernible. For some little time after the pupal moult the pupa is sticky to the touch owing to the moulting fluid and there is a palpitation on the vertex combined with frequent movements of the abdomen. The new pupal integument

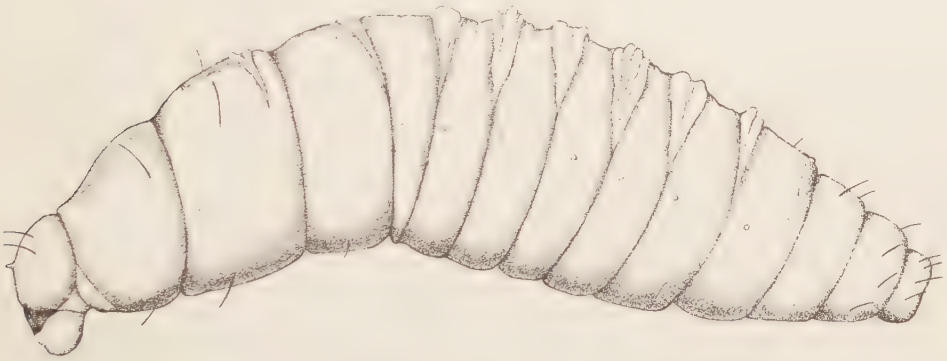

Fig. 10. The larva of *Harmolita* in the stage immediately preceding the semi-pupal stage ×300 diams.

is very soft and flexible, but the general conformation of the chief sclerites of the adult can be seen. The pedicel or "waist" is marked only by a very slight constriction and the segmentation of the abdomen is very faint.

There is a well-defined ridge on the head just ventral to the insertion of the antennae, and beneath this ridge is a swelling which represents the

rudiments of the labium. Below the latter comes the rudiments of the mandibles followed by the maxillae and labium with their palps. The legs and antennae are glued to the ventral surface but their segmentation is very indistinct, only the limits of the coxae on the legs and the scapes on the antennae being demarcated.

The male pupae are easily separated from the female by reason of their markedly longer antennae. The mesothoracic wing cases are glued against the body and hide the metathoracic wing cases. The compound eyes are present just after pupation but no ocelli are visible at this stage. A pronounced lateral ridge is present on each side of the abdomen which extends from the propodeum to the posterior extremity of the abdomen.

At about four days old the pedicel becomes better defined owing to an increase in the constriction; the cuticle becomes harder and indications have appeared of the two dorsal ocelli. The darkening of the pupa begins on the eighth day of the pupal stage. A dark brown streak appears in the median line near the mouth region. Soon afterwards black pigment is deposited on the compound eyes, on the mandibles, on the intersegmental regions of the abdomen, and on the three ocelli which are now visible. This blackening process continues until by the sixteenth day of pupation the pupa is black in colour. Meanwhile the pedicel becomes still more constricted and the segmentation of the legs and antennae more distinct. Chitinisation of the imaginal integument proceeds and the outer pupal coat becomes dry and loosely attached to the hardening imaginal coat beneath.

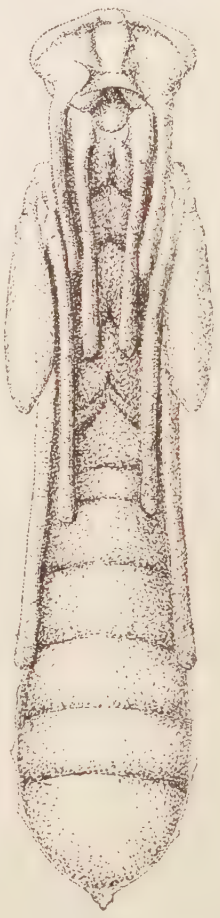

Fig. 11. The pupa about the middle of the pupal period × 50 diams.

The length of the pupal period is about 40 days, but this can be reduced to 28 days by allowing pupation to take place in an artificially heated laboratory.

11. Natural Enemies.

Although many galls produced by *Harmolita graminicola* have been examined, only one species of insect parasite was discovered and its degree of infestation would not exceed 1 per cent. This parasite attacks the larval stage and it was determined as *Bracon erythrostictus* (Lyle) (Fig. 8).

The larva of *Harmolita* is parasitised at an early stage within the dried skin of the larva which assumes a brownish colour (Fig. 9). *Bracon*

Fig. 12. View of the galls made by larvae of *Harmolita* on *Triticum repens* about natural size.

erythrostictus emerges about a fortnight to three weeks before *Harmolita* appears. The former parasitises both species of *Harmolita* dealt with in this paper. The parasites of *Harmolita* are recognised as of great economic value in the United States of America as they help very largely to keep jointworm in check in normal years.

The only other natural enemy of any importance were certain birds. Large numbers of larvae are pecked out of galls by birds, particularly by tits; especially if the winter is severe.

12. Summary.

This paper is a contribution to our very scanty knowledge of the British *Harmolita*, a phytophagous genus of the superfamily Chalcidoidea (Hymenoptera). Many species of this genus are pests on cereals and cultivated grasses; one species, *Harmolita tritici*, is the notorious joint-worm of America and Russia, whilst species attacking many other cereals and grasses have been recorded in Europe and America. An account is here given for the first time of the life history and biology of a British species of *Harmolita*, namely *Harmolita graminicola*, which is a gall former on couch grass (*Triticum repens*).

The larva of another undetermined species of *Harmolita*, also gallicolous on *Triticum repens* is figured and briefly described.

The adult of *Harmolita graminicola* begins to emerge in the last week of June and continues to appear during the first week of July. When the inflorescence has appeared through the sheathing leaves the female fly will not lay eggs on them. One egg is deposited on each culm just beneath the rudimentary inflorescence, the ovipositor of the fly piercing the sheathing leaves in order to reach the desired position. The *Harmolitae* of both species were bred in captivity and the length of the period of incubation of the egg was found to be between three and four weeks. Experiments were conducted to ascertain whether parthenogenesis occurred in this species. The results were negative although perhaps not numerous enough to be conclusive.

The larva possesses four instars and moults three times whilst feeding, another moult occurring at pupation. The larval instars can be differentiated most exactly by the state of development of the sub-apical tooth on the mandible. The sub-apical tooth is absent from the mandibles of the first instar; only incipiently developed in the second instar; better developed in the third and fully formed in the fourth instar. The larva becomes full fed in October and hibernation takes place in the larval stage. Pupation begins to occur near the end of April but the great majority pupate in the middle of May.

There is a short semi-pupal stage of from 12 to 24 hours duration immediately before the pupal moult takes place. The pupal stage lasts about 40 days and there is only one generation each year. The larva of *Bracon erythrostictus* (Lyle) was found to be parasitic on *Harmolita* in the latter's larval stages; no other insect parasites were found. Both *Harmolita graminicola* (Gir) and the undetermined species refused to breed on wheat or any other plants of the *Triticum* genus although many attempts were made to induce them to do so.

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REVIEWS

The Theory of the Gene. By Thomas Hunt Morgan. Yale University Press, 1926. Pp. xvi + 343, Figs. 156. 18*s.* net.

This volume is an outline of the theory of genetics from the point of view of the American worker on *Drosophila*. Its wide scope is shown by the chapter headings which are as follows: I. The fundamental principles of genetics. II. Particulate theories of heredity. III. The mechanism of heredity. IV. Chromosomes and genes. V. The origin of mutant characters. VI. Are mutant recessive genes produced by losses of genes? VII. The location of genes in related species. VIII. The tetraploids, or fourfold type. IX. Triploids. X. Haploids. XI. Polyploid series. XII. Heteroploids. XIII. Species crossing and changes in chromosome number. XIV. Sex and genes. XV. Other methods of sex-determination involving the sex-chromosomes. XVI. Intersexes. XVII. Sex reversals. XVIII. Stability of the gene. XIX. General conclusions. Bibliography. Index.

The chromosome interpretation of the mechanism of heredity is accepted throughout and fresh data in support of it are brought forward. Chapters I–IV are essentially introductory. Chapter V is all too short for the critical importance of its subject and leaves one with a curiously unsatisfied feeling. It is a pity that the opportunity was not taken to give an exact and unequivocal statement of what the term "mutation" connotes. So much controversy has arisen from the different usages of this word, and there are such varied and discrepant definitions of it in recent genetical treatises, that a precise statement by Prof. Morgan, which, one might expect would be adhered to by American workers, would do much to clear up the confusion.

Chapter VI in which the "Presence and Absence Theory" is discussed adversely is of considerable interest, but certain data such as those relating to the albino guinea-pig, the recessive black-rabbit, the white factor in the jungle-fowl and the bar-eye in *Drosophila* are capable of interpretations other than those adopted.

Chapters VII–XIII dealing with various aberrant and multiple chromosome arrangements are a masterly summary of the very widely scattered data in this field. One expected however to find some reference to the recent criticisms of Jeffrey and Hicks, who are not mentioned, and of Lotsy to whom only two references, neither later than 1916, are given.

Chapters XIV–XVII, in which problems of sex are considered, are perhaps the most interesting portion of the volume although not so coherent in treatment as the previous section. The discussion of intersexes and sex-reversals in both animals and plants is particularly useful. It may perhaps be still premature, but one would have liked to see included reference to the striking cases of sex-reversals in fungi.

Chapter XVIII on the stability of the gene is exasperatingly brief and gives little idea of the present position or perspective of the problem. One would have liked a much fuller discussion of the "inheritance of acquired characters," more particularly from the plant aspect; to find reference to Kammerer, Tornier, Guyer, etc.; to see some discussion of this question in relation to bacteria, fungi and protozoa, where it is of primary importance and where evidence is rapidly accumulating. The causes or stimuli of changes of gene are still as obscure as ever in the higher forms, but, in micro-organisms, they seem to be more within our reach. The data from all fields of study, however, seem to point more and more clearly to the view that "mutation" is no fortuitous happening but is definitely causal in nature, a specific and orderly process.

The present book constitutes the nineteenth volume published on the Silliman Lectures Foundation of Yale University. The only other book in this series of general

biological interest is the eighth volume which is Bateson's *Problems of Genetics*, published in 1913. If one compares these volumes one obtains a vivid impression of the changes in sheer amount of data (almost the whole of the *Drosophila* work) in values and in perspective that thirteen years of intense genetical research have produced. Also, incidentally, one cannot help being struck by the difference between the big philosophic outlook and synthetic grasp of Bateson's volume, and the widely ranging but far less coherent and more intensive outlook of Morgan's volume.

Viewing the present book in the light of the past thirteen years of research, which is just one-half the age of modern genetics, one cannot help feeling that fresh points of view are needed in this field of work. For twenty-six years genetic research has been almost entirely a matter of cytological detail and of juggling with Mendelian factors in experimental breeding: the physiological bases of the genotype and the environmental conditioning of the phenotype have been neglected. In very few genetics' laboratories are there first-class physiologists at work and yet these could do much to widen the present canalised approaches to genetical problems and to drive new avenues.

The volume contains a very useful bibliography which would have been still more useful if it contained references to more of the authors mentioned by name in the text. For example—and this is only one of many in the book—in discussing *Tetranychus bimaculatus* the references given are "(Perkins, H. A. Morgan, Bank, Ewing, Parker)," not one of whom is mentioned in the bibliography. The volume also apparently contains errors on pages 12, 113 and 293 and in Figs. 11, 12 and 141, but otherwise it is beautifully produced, both printing and binding, and the illustrations are admirable.

The Theory of the Gene is an up-to-date presentation of the views of one of the most eminent of living geneticists and taken in conjunction with his *Mechanism of Mendelian Heredity* (1923), and his recent detailed monograph on the *Genetics of Drosophila* (1925), it gives one a very good idea of the present position of the monumental researches that are steadily taking shape in the Columbia laboratories and of the lines of thought that are crystallising out in them. No research worker in applied biology can afford to neglect these more theoretical aspects of genetics, for they underlie all practical problems of disease, of crop growth, animal husbandry, economic breeding and the like.

WILLIAM B. BRIERLEY

Life of Plants. By Sir Frederick Keeble. Clarendon Press, Oxford, 1926. Pp. 256, Figs. 51. 5*s*. net.

Since *Chapters in Modern Botany* by Geddes, the only botanical pocket volumes that one remembers as worth while are Praeger's *Open Air Studies in Botany*, the volumes by Scott and Farmer in the Home University Library, the Cambridge Manuals by Seward and Bower and Dixon's lectures. The present volume bears comparison with these. It is packed full of meat and yet so easily is it written and so apt are the allusions and illustrations that the reader is almost unaware of the strength of his diet. *Life of Plants* is an Essay in Botanik rather than a botanical text-book and presents essentially a personal point of view. Many a botanist might quarrel with the author's perspective and balance of values, his avoidance of disease and death which are a too often neglected aspect of plant life, the little attention given to the dominance of gametophyte or sporophyte phases in different plant groups, the confident acceptance of hormones to explain unification of behaviour, the neglect of the more "social" aspects of plant life and so forth. That one finds so many points on which to differ is a tribute to the unusual and stimulating quality of the book. The volume is finely printed and bound and the illustrations are refreshingly original.

WILLIAM B. BRIERLEY

Contributions from the Harvard Institute for Tropical Biology and Medicine, III, 1926. Cambridge (Mass.): Harvard University Press; London: Humphrey Milford, Oxford University Press. 4to; 110 pp. and 4 Plates. Price 11*s.* net. I. Report on Sugar-cane Borers at Soledad, Cuba, by G. Salt. II. Dry-Season Studies of Cane Homoptera at Soledad, Cuba, by J. G. Myers.

The first of the above articles deals with observations on sugar-cane borers made during a period of about ten weeks at Soledad, Cuba. As might be expected, the brevity of the time devoted to so comprehensive a subject did not allow for any very detailed studies to be made. Although there are in Cuba a number of scientific workers engaged upon various problems, very few are entomologists, and the need for information respecting borer attacks is urged as the excuse for what would otherwise have been regarded as a too hastily compiled publication. It appears that the 1924–25 sugar-cane crop at Central Soledad was infested by moth borer (*Diatraea saccharalis*) to the extent of 18·5 per cent. of all canes arriving at the mill. It is claimed that actual counts made at the mill are more reliable than field counts involving the same amount of time and work. There is, however, a wide range in the percentage of infestation of this insect in the various colonias or plantations: in one colonia only 5·7 per cent. infestation is recorded while the highest infestation amounted to 33·36 per cent. The distribution of the attacks appears to be influenced by topography, hills being infested to a lesser degree than valleys, and the high-land colonias show a lower percentage of attack than low-lying colonias. As an explanation it is suggested that since the *Diatraea* seems to have a preference among wild plants for aquatic grasses, the physiological state of sugar-cane in low lands and valleys more nearly simulates that of such grasses and suffers heavier depredation in consequence. In view of the fact that natural control by means of parasites, although definitely beneficial, does not sufficiently dominate the situation, various control practices are recommended. Of the several measures advised it is interesting to note that the burning off of infested cane fields before cutting, and also of the trash after cutting, is recommended to be discontinued. It has been shown in Louisiana that trash burning is very detrimental in destroying great numbers of a beneficial egg-parasite without any commensurate advantage resulting, and the same is assumed to be true in Cuba. A list of parasites is given at the end of the article and the most important is the Tachinid *Lixophaga diatraeae* which, it may be added, has been purposely introduced from Cuba into other sugar-growing countries. Among other cane-borers, the weevil borer (*Metamasius sericeus*) appears to be more serious than was previously supposed, while on the other hand injuries occasioned by *Xyleborus* sp. and by Termites are considered to be of little importance.

The second article is likewise the result of a brief sojourn at Soledad and is written by Mr J. G. Myers. His object was to study those members of the suborder Homoptera involved, or possibly involved, as carriers of mosaic disease of cane and allied grasses. The article is intended only as a prelininary study of the situation as it appears in the driest season of the year. Our present knowledge of cane mosaic transmission by insects is summarised, and it may be said that the corn leafhopper can transmit mosaic from corn to corn but does not occur in cane. *Aphis maidis* can carry the disease from other grasses to cane, and the aphid *Carolinaia cyperi* transmits the disease from sedge to cane. None of these occur normally on cane but the two last-mentioned species have been shown to migrate to cane when their weed host-plants are eliminated. The question of insect vectors has not received much attention at Soledad where it appears that *Aphis maidia* is very rare. Whether there is any other factor than diseased seed-pieces to explain most of the present distribution of mosaic at Soledad is to the author an open question. He discusses the evidence for the incrimination of other cane Homoptera and finds that there are only nine species that, under dry-season conditions, can properly be regarded as true cane inhabiting forms. There appears to be no evidence that any of these function as vectors of mosaic disease.

A. D. IMMS

Plant Nutrition and Crop Production. By E. J. Russell. Cambridge University Press, 1926. Pp. ix + 115 with 21 Plates and 37 Text-figures. Price 12*s.* 6*d.* net.

The present volume embodies the Hitchcock lectures delivered in the University of California by Sir John Russell in 1924.

The subject matter is divided into five chapters entitled respectively, The study of plant nutrients; Positive science and exact demonstration; Decay and the living plant; The soil micro-organisms; The soil and the living plant. In the first of these the author traces the history of the study of plant nutrition from the early speculations of Thales (600 B.C.) and the equivocal experiments of Van Helmont (1620) to modern times. It was not till the dawn of the nineteenth century that the modern era of exact knowledge may be said to have begun with the publication of de Saussure's *Recherches chimiques sur la Vegetation.* In 1834 Boussingault initiated exact field experiments, quickly followed by Liebig's valuable generalisations, in which the capacity of plants to utilise simple organic compounds was for the first time adequately emphasised. It was Liebig's work that inspired Lawes' experiments with artificial manures, which demonstrated the importance of phosphates and nitrogenous compounds and led to the commercial production of superphosphate.

The development of research on manurial treatment, the problems of increased yield in crop production, are here presented in the clear and logical manner of which the author is a past master. By the careful selection of detail the reader is led to the realisation of the full complexity of the problem when we attempt to grow two blades where one grew before. Far from being simple the process involves the modification of a whole chain of conditions and phenomena, in which success is dependent on the strengthening of each individual link. We are concerned not merely with the direct reaction of the species to a particular treatment, but with its effect on the associated organisms both macroscopic and microscopic, its effect on the physical and chemical constitution of the soil and on the manner in which the soil itself and the crop it bears reacts to the climatic environment. Not the least important of the variables with which we interfere is the organisation of the individual plant, the reaction of whose internal environment is modified by the changed external conditions.

The striking differences which organisms of the same genotypic constitution may exhibit in their phenotypic expression, as witness the development or non-development of supernumerary legs in the fruit fly according to the temperature of their environment, opens up a wide vista of possibilities over and above the selective action which manurial treatments and soil-amendments will inevitably exert upon the numerous genotypic strains which comprise a cultivated race, and which may only be capable of selection by physiological means.

The author recalls the old controversy regarding the relative merits of artificial fertilisers and farmyard manure, both of which are now recognised to have their appropriate place in agricultural economy. Though we have travelled far in the short period since Lawes and Gilbert conducted their first experiments, yet how great a distance has yet to be covered is shown by the wastefulness of our existing methods, strikingly illustrated in the balance sheet of calories for the Broadbalk field where, to produce food for two men, energy sufficient to feed twelve is dissipated! Further, the delicacy of the balance which has to be maintained is indicated by the comparative constancy of the carbon-nitrogen ratio under equivalent climatic conditions. Fertilisers or partial sterilisation are means of restoring the appropriate balance between chemical constituents on the one hand and micro-organisms on the other; indeed it may be questioned whether if such treatment permanently upset the balance, the consequences might not be as drastic as those which have followed the artificial disturbances by man of the balance of nature in Australia and New Zealand.

Sir John whilst rightly stressing the complexity of the problems involved indicates the lines along which further progress may be looked for, and in particular the need for investigations on the lines of pure science unhampered by the trammels that

enmesh the empiricist and technologist. In no direction may greater advance be looked for than in our knowledge of the micro-organic population of the soil, and the author envisages the time when the soil fauna and flora of cultivated lands shall be tamed in the service of man.

It would demand a volume many times the size of this work to epitomise the achievements in agricultural research, but the reader of these pages will obtain a clear idea of the trend of modern investigation and the value of pure science to the practical man. If proof of this latter were needed, no better example could perhaps be cited than the electrically charged ploughshare which appreciably reduces the frictional resistance of the soil, for this was a direct outcome of the study of soil colloids than which no subject, superficially, would appear more academic or more widely separated from the immediate interests of the farmer.

E. J. SALISBURY

Citrus Diseases and their Control. By H. C. Fawcett and H. A. Lee. McGraw-Hill Book Co., 1926. Pp. 582. Figs. 205 (15 coloured). 25*s.* net.

Plant pathology is arriving at the stage when the amount of data available is so great that special treatises on diseases of particular host plants, or on particular aspects of disease, are not only desirable but necessary. We have had such early volumes as that of Delacroix on *Les Maladies et les ennemis des Cafeiers*, or of Watt on *The Pests and Blights of the Tea Plant*, and more recently Hiley's *Fungal Diseases of the Common Larch*, Petch's *Diseases of the Tea Bush* and *Diseases and Pests of the Rubber Tree*, but the present work by Fawcett and Lee on *Citrus Diseases and their Control* sets a new standard in such publications. The book itself runs to nearly 600 closely packed pages, and even so, does not include diseases due to insect pests.

The volume is divided into four parts. The first seven chapters are devoted to general considerations such as the history of citrus-disease investigations and the structure and physiology of citrus; types of citrus diseases and the inevitable outline classification of fungi; the geographical distribution of citrus diseases; conditions affecting severity and distribution of citrus diseases; general principles of prevention and treatment of citrus diseases; fungicides, disinfectants, paints and waxes; cultural operations in relation to citrus diseases.

This first portion is very unequal in treatment as though the authors had not clearly in their minds the audience they were writing for. What does stand out, however, in this very interesting discussion, is the empirical nature of much of this general knowledge, and the little we know, more particularly, of the geographical distribution of disease and of its climatic and cultural relationships. It is, of course, extremely difficult and often impossible to collate such generalised pictures from scattered publications, partly because of the different degrees of accuracy of work in different countries, and partly because in some countries the requisite workers are absent, or where they are present, the work is often done or described from such different points of view that there are few comparable features. The collaboration in the present volume of two authors of unusually wide experience has enabled them to surmount many of these difficulties. Nothing can be more valuable in this respect than the practice increasingly adopted by American specialists of visiting the different countries in which some particular crop or disease occurs, and so enabling a comparative survey of the whole situation to be made by one pair of eyes. Such a system could be most usefully grafted on to our own practice in the overseas dominions and colonies. The need is met to a certain extent in our Imperial Bureau of Mycology, which functions most efficiently as a central information bureau, exchange and clearing-house, but a vast amount of good would result, if there was more free movement and exchange between mycologists themselves in the colonies and overseas dominions. Such a visiting, loan or exchange system, would of course be very difficult to administrate, but within the British Empire the opportunities are unequalled and the advantages accruing would far more than compensate for the effort.

To return to our volume, the three remaining portions are a straightforward account of the diseases of the citrus tree; part II, dealing with root and trunk diseases; part III, with diseases of branches, twigs and leaves, and part IV, with fruit diseases. It is interesting to note how many of the diseases of roots and trunk and of fruit are caused by soil-dwelling fungi. This is an aspect of plant pathology that has been vaguely realised for some time, but only recently has it assumed a prominent place. There is very little doubt that the soil is a great reservoir of disease-causing fungi, which, in numerous cases can live for long periods if not indefinitely in a saprophytic state, becoming parasites when the opportunity offers. Some years' experience of such work leads one to suspect that there are very few parasitic fungi indeed, which, able to live saprophytically, cannot sooner or later be isolated from soil. The number of different kinds of parasitic fungi present in soil is legion, and it is only a question of applying suitable methods in order to isolate them. Not only in citrus diseases, but in diseases of all crops, far more attention must in future be paid to soil microbiology.

Parts II, III and IV of the book are a most adequate summary of our knowledge, and the authors have succeeded in occupying the position defined in their preface that they "have attempted to present in this book a discussion of the present information on citrus diseases occurring in all parts of the world where citrus fruits are grown." In the consideration of all diseases spraying and cultural treatments are recommended where these are known and feasible, but the emphasis throughout is laid on the value of growing resistant or immune varieties as the only permanent and really satisfactory method of disease control.

One of the best chapters in the volume is the last on "General Problems of Deterioration and Decay of Citrus Fruit," and this points the way to developments of plant pathology to which, in this country, far more attention should be given. We are too apt to think that the work of the plant pathologist stops when the field crop is gathered, whereas it only really stops when the produce is consumed. Storage, transport, and markets' pathology are hardly known here and present a large and promising field of study.

Considering the size and nature of the book the consistently high level of writing is unusual. One cannot help liking the early-Victorian savour of the term "Internal Decline of Lemons" or appreciating the description of sour rot as "A putrid, sour, dirty, leaking, maggot-filled mass of mud." There is however loose wording on pages 118, 302, 362 and 422, and misprints have been noticed on pages 37, 38, 98, 113, 198, 276, 311, 354, 373, 506, 543 and 580.

The book is illustrated by 190 text-figures, many of the photographic reproductions being curiously flat, and occasionally (*e.g.* fig. 82 D), frankly bad. In Figure 153, the "A" and "B" referred to in the legend are not marked in the print. There are also 15 plates which contain some of the best colour photographs of plant diseases yet published. The bibliography runs to 18 pages and is an extremely useful and accurate compilation; the index is full and convenient to use. The actual format of the book has the technical excellence of all the volumes in this fine series.

The preparation of the volume, in which so much of the matter is the outcome of the authors' personal researches, is a very fine achievement, and sets a standard that will be difficult to equal. The book will immediately take rank as the authoritative and indispensable work of reference on the subject.

WILLIAM B. BRIERLEY

OBITUARY

Prof. F. W. GAMBLE, F.R.S.

Prof. F. W. Gamble, D.Sc., F.R.S., died at Alvechurch, Worcestershire, on September 14th last at the age of 57. Born at Manchester in 1869 he was the son of the late Mr William Gamble of Arnside, Westmoreland. His early education was at the Manchester Grammar School and from there he passed on to the University of that city. It was here he came under the influence of the teaching of the late Prof. Milnes Marshall, and Gamble proved one of his most successful pupils. His university studies culminated in his graduating with first class honours in zoology in 1891, and his appointment to a Berkeley Fellowship in the following year. After a period of study at Leipzig University he returned to Manchester in order to take up the post of lecturer in zoology. Gamble was a sound and successful teacher in his *alma mater*, and this had much to do with his subsequent appointment (in 1909) to the chair of zoology at Birmingham University, a post which he held up to the time of his death. In his early years as a University teacher he accomplished useful research work, and his first important memoir was on British Marine Turbellaria, which appeared in 1893. This work was followed by several researches of outstanding merit carried out in conjunction with colleagues. Mention needs to be made of the fine memoir on *Arenicola*, prepared in conjunction with Prof. J. H. Ashworth, and his experimental studies on the colour changes of *Hippolyte* and on the bionomics of *Convoluta*, both works being carried out in collaboration with Sir F. W. Keeble. After his appointment to Birmingham heavy teaching duties fell upon Prof. Gamble, and for a time he also acted as director of a small school of agricultural helminthology which had been established, in conjunction with his department, by the Ministry of Agriculture. For a short period he served as a member of the Research Council of that same Ministry. Prof. Gamble was also a member of the Council of the Association of Economic Biologists at the time of his death, but ill-health was responsible for his inability to attend its meetings. To the general student he was perhaps best known as the editor of several successive editions of Marshall and Hurst's well-known *Practical Zoology*. In 1907 he was elected an F.R.S., and in 1924 he presided over the Zoology Section of the British Association at Toronto. Prof. Gamble was married in 1904 and his widow survives him: he leaves no children.

A. D. I.